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PER- and POLYFLUORALKYL SUBSTANCES SAMPLING AND ANALYSIS QUALITY ASSURANCE PROJECT PLAN

OPERABLE UNIT THREE

W.R. GRACE SUPERFUND SITE
ACTON, MASSACHUSETTS

PREPARED FOR

W.R. GRACE & CO. - CONN
7500 GRACE DRIVE
COLUMBIA, MARYLAND 21044

PREPARED BY

TETRA TECH, INC.
3 LAN DRIVE, SUITE 100
WESTFORD, MASSACHUSETTS 01886
(978) 303-8531

TETRA TECH

JUNE 14, 2019

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ACRONYMS*

AFFF	Aqueous film forming foam
ATSDR	Agency for Toxic Substances and Disease Registry
COC	Contaminant of Concern
FRB	Field Reagent Blank
FSP	Field Sampling Plan
Grace	W.R. Grace & Co. – Conn.
ISCR	Initial Site Characterization Report (HIS GeoTrans, 1998)
MassDEP	Massachusetts Department of Environmental Protection
ng/L	Nanograms per liter
OU	Operable Unit
PFAS	Per- and Polyfluoroalkyl Substances
PFOA	Perfluorooctanic Acid
PFOS	Perfluorooctanesulfonic Acid
POP	Project Operation Plan
PPT	Parts Per Trillion
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QC	Quality Control
RCRA	Resource Conservation and Recovery Act
RD/RA	Remedial Design/Remedial Action
RI/FS	Remedial Investigation/Feasibility Study
SDG	Sample Delivery Group
Site	W.R. Grace & Co. – Conn. Superfund Site
SMP	Site Management Plan
SOP	Standard Operating Procedures
SOW	Statement of Work
US EPA	United States Environmental Protection Agency
VDC	1,1-dichloroethene

Acronyms for PFAS specific compounds are listed on Table 3-10

EXECUTIVE SUMMARY

This Quality Assurance Project Plan (QAPP) has been prepared to accompany the Field Sampling Plan (FSP) for Per- and Polyfluoroalkyl Substances (PFAS) in June 2019 at the W. R. Grace & Co. – Conn. (Grace) Acton Superfund Site (the Site). The FSP and QAPP incorporate by reference standard operating procedures (SOPs) and other components of the 2000 Project Operations Plan (POP)(March 10, 2000) and amendments submitted in 2007 (February 1, 2007).

The original POP was prepared to address the Contaminants of Concern (COCs) as determined in the Consent Decree Scope of Work (SOW) for the site. The COCs include: benzene, VDC (vinylidene chloride or 1,1-dichloroethene), and vinyl chloride. The 2000 amended POP was amended in 2007 to address sampling and analysis for 1,4-dioxane, which is an emerging contaminant and not a COC and Remedial Design/Remedial Action (RD/RA) activities. This QAPP is to support the FSP and supplement the March 2000 POP for another emerging contaminant, PFAS.

This document describes procedures and criteria for defining DQOs, method of analysis, project required reporting limits, and measurement performance criteria for accuracy, precision, representativeness, completeness, comparability, and sensitivity for PFAS. This amendment is required because this is the first time PFAS will be analyzed for at the Grace site.

The structure of this document is to mirror each of the current March 2000 QAPP's sections to note only changes necessary to address PFAS compounds, without repeating unchanged information. No changes after Section 9 of the original 2000 QAPP are necessary.

1 PROJECT DESCRIPTION

No changes.

2 ANALYTICAL DATA METHODOLOGY, REPORTING LIMITS AND MEASUREMENT CRITERIA

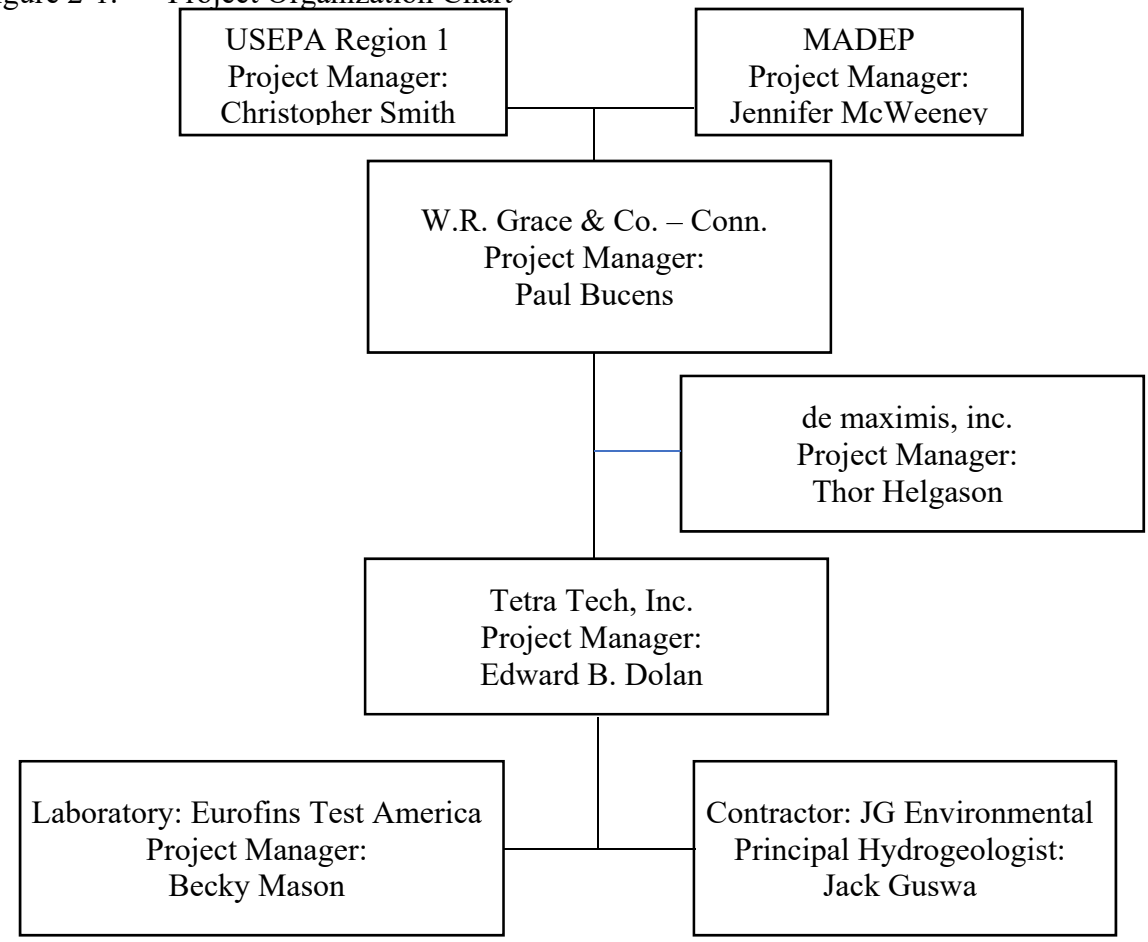
The following are changes to the project team contractors and project organization chart.

Table 2-1. Project Team Contractors

PROJECT COORDINATOR
CONTRACTOR: TETRA TECH, INC.
PROJECT MANAGER: Edward B. Dolan
3 Lan Drive, Suite 100
Westford, MA 01886
Phone: 978-303-8531

TECHNICAL CONSULTANT
CONTRACTOR: JG ENVIRONMENTAL, INC.
PRINCIPAL HYDROGEOLOGIST: Jack Guswa, Ph.D., CPHg
20 Bridge Road, Unit 7
Florence, MA 01062-1096
Phone: 413-584-1830

Figure 2-1. Project Organization Chart



3 QUALITY ASSURANCE OBJECTIVES

The following presents additional tables referenced within report section and sub-sections.

Table 3-9 (analogous to original QAPP Table 3-1) provides the analytical Data Quality Objectives for PFAS.

Table 3-9. Summary of PFAS Analysis Data Quality Objectives for Water Samples	
DQO Parameter	Objectives
Precision	Table 3-10
Accuracy	Table 3-10
Representativeness	PFAS Analytes: ≤ 30% RPD
Completeness	90%
Comparability	Based on Precision, Accuracy, and Analytical Method
Notes: RD= Relative Percent Difference	

Table 3-10 (analogous to original QAPP table 3-2 through 3-4) provides detail regarding the specific precision and accuracy objectives for PFAS analysis.

Table 3.10 Specific Analytical Laboratory Data Quality Objectives for Precision and Accuracy for PFAS Standard List for Method 537M

Analysis Group: Groundwater
Method Description: PFAS, Standard List (24 Analytes)
Method Code: 537 Modified
Preparation Method: 3535_PFC

Parameter	QC Compounds	Field Duplicate Precision (RPD)[a]	MS/MSD Precision (RPD)	Blanks*	LCS & MS/MSD Accuracy (% Recovery)	Surrogate Accuracy (% Recovery)
PFAS	Perfluorobutanoic acid (PFBA)	<30%	<30%	≤ Reporting Limit	70 - 130	N/A
	Perfluoropentanoic acid (PFPeA)				66 - 126	
	Perfluorohexanoic acid (PFHxA)				66 - 126	
	Perfluoroheptanoic acid (PFHpA)				66 - 126	
	Perfluorooctanoic acid (PFOA)				64 - 124	
	Perfluorononanoic acid (PFNA)				68 - 128	
	Perfluorodecanoic acid (PFDA)				69 - 129	
	Perfluoroundecanoic acid (PFUnA)				60 - 120	
	Perfluorododecanoic acid (PFDoA)				71 - 131	
	Perfluorotridecanoic acid (PFTriA)				72 - 132	
	Perfluorotetradecanoic acid (PFTeA)				68 - 128	
	Perfluorobutanesulfonic acid (PFBS)				73 - 133	
	Perfluoropentanesulfonic acid (PFPeS)				70 - 130	
	Perfluorohexanesulfonic acid (PFHxS)				63 - 123	
	Perfluoroheptanesulfonic Acid (PFHpS)				68 - 128	
	Perfluorooctanesulfonic acid (PFOS)				67 - 127	
	Perfluorononanesulfonic acid (PFNS)				70 - 130	
	Perfluorodecanesulfonic acid (PFDS)				68 - 128	
	Perfluorooctanesulfonamide (FOSA)				70 - 130	
	N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)				67 - 127	
	N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)				65 - 125	
	4:2 FTS				70 - 130	
	6:2 FTS				66 - 126	
	8:2 FTS				67 - 127	

Notes:
blanks include field equipment blanks, field duplicate and field reagent blanks
N/A=Surrogates are used to evaluate the effects of sample matrices and extraction recovery. With isotope dilution analysis, each sample is spiked with isotopically labeled surrogates which provide data on matrix effects and any potential extraction losses for each individual sample.

Table 3-11 (analogous to original QAPP Table 3-8) lists the DQO Level for PFAS data validation.

Table 3-11. DQO Levels for PFAS Analysis		
Parameter	Field (F) or Laboratory (L)	DQO Level
PFAS	L	IV

Notes: Data Quality Objective (DOQ) Levels are based on those defined by USEPA in "Data Quality Objectives for Remedial Response Activities", USEPA/540/G-87-003. Definitions applicable to DQO levels for this project are as follows:

- Level IV= CLP-like. This level is characterized by rigorous QA/QC protocols and documentation and provides qualitative and quantitative analytical data.
- level III = This level is used primarily in support or engineering studies using standard EPA approved procedures. Limited documentation required.
- Level I = Field screening. This level is characterized by the use of portable instruments which can provide real-time data to assist in the optimization of sampling point locations and for health and safety support. Data can be generated regarding the presence or absence of certain contaminants (especially volatiles) at sampling locations.

4 FIELD PROCEDURES

The field procedures for PFAS sampling vary significantly from those described in the original 2000 QAPP and the 2007 amendments. Therefore, field procedures located in the PFAS Field Sampling Plan should be used.

Below is Table 4-2 (analogous to Table 4-1 in the original QAPP) presents sample preservation and holding time information for PFAS samples.

Table 4-2. PFAS Sample Preservation and Holding Time

Matrix	Parameter	Sample Container(s)	Preservative	Holding Time
Groundwater, Rinsate Blanks and FRBs	PFAS	250 mL HDPE. No Teflon-lined caps.	$\leq$10°C; protect from light.	Extraction: 14 days Analysis: 28 days

Notes:
FRB=field reagent blank
HDPE= high density polyethylene

5 SAMPLE CUSTODY

No changes.

6 CALIBRATION PROCEDURES AND FREQUENCY

A change to the text to include PFAS as a Level IV DQO.

7 ANALYTICAL PROCEDURES

The following information is to be added to this section to include PFAS analytical procedures.

The Sacramento, CA laboratory for Test America (Eurofins TestAmerica) will use a method that is an enhanced method 537.1 and would be compliant with the pending EPA method (as currently planned). This method is "537M Manual SPE" which is applicable for non-potable water (groundwater and surface samples) and includes isotope dilution to filter out potential interferences to improve measurement accuracy. The method will report the 14 analytes referenced in the MADEP June 19, 2018 Fact Sheet, as well as the additional 10 analytes that are listed in the pending USEPA "non-potable" method (EPA Method 8328).

The isotope dilution technique is employed with this method for the compounds of interest. The isotope dilution analytes (IDA) consist of carbon-13 labeled analogs, oxygen-18 labeled analogs, or deuterated analogs of the compounds of interest, and they are spiked into the samples at the time of extraction. This technique allows for the correction for analytical bias encountered when analyzing more chemically complex environmental samples. The isotopically labeled compounds are chemically similar to the compounds of concern and are therefore affected by sample-related interferences to the same extent as the compounds of concern. Compounds that do not have an identically labeled analog are quantitated by the IDA method using a closely related labeled analog.

Table 7-7 below (analogous to original QAPP Table 7-1) presents the analytical method to be used for analyzing water samples for PFAS.

Table 7-7. PFAS Laboratory and Field Analytical Methods

Parameter	Method	Method Reference
PFAS	537M (Modified) WS-LC-0025	[1]

Notes: [1] US EPA, "Method 537 - Determination of Selected Perfluorinated alkyl acids in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometery (LC/MS/MS)", Version 1.1, September 2009, J.A. Shoemaker, P.E. Grimmett, B.K. Boutin, EPA Document #: EPA/600/R-08/092

Table 7-8 (analogous to original QAPP Tables 7-2 through 7-6) presents the PFAS analyte list and reporting limits.

Table 7-8. PFAS Analyte List, Reporting Limit, and Method Detection Limit

Analyte	CAS Number	RL	MDL
Perfluorobutanoic acid (PFBA)	375-22-4	2.00	0.35
Perfluoropentanoic acid (PFPeA)	2706-90-3	2.00	0.49
Perfluorohexanoic acid (PFHxA)	307-24-4	2.00	0.58
Perfluoroheptanoic acid (PFHpA)	375-85-9	2.00	0.25
Perfluorooctanoic acid (PFOA)	335-67-1	2.00	0.85
Perfluorononanoic acid (PFNA)	375-95-1	2.00	0.27
Perfluorodecanoic acid (PFDA)	335-76-2	2.00	0.31
Perfluoroundecanoic acid (PFUnA)	2058-94-8	2.00	1.10
Perfluorododecanoic acid (PFDoA)	307-55-1	2.00	0.55
Perfluorotridecanoic acid (PFTriA)	72629-94-8	2.00	1.30
Perfluorotetradecanoic acid (PFTeA)	376-06-7	2.00	0.29
Perfluorobutanesulfonic acid (PFBS)	375-73-5	2.00	0.20
Perfluoropentanesulfonic acid (PFPeS)	2706-91-4	2.00	0.30
Perfluorohexanesulfonic acid (PFHxS)	355-46-4	2.00	0.17
Perfluoroheptanesulfonic Acid (PFHpS)	375-92-8	2.00	0.19
Perfluorooctanesulfonic acid (PFOS)	1763-23-1	2.00	0.54
Perfluorononanesulfonic acid (PFNS)	68259-12-1	2.00	0.16
Perfluorodecanesulfonic acid (PFDS)	335-77-3	2.00	0.32
Perfluorooctanesulfonamide (FOSA)	754-91-6	2.00	0.35
N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	2355-31-9	20.0	3.10
N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	2991-50-6	20.0	1.90
4:2 FTS	757124-72-4	20.0	5.20
6:2 FTS	27619-97-2	20.0	2.00
8:2 FTS	39108-34-4	20.0	2.00
ng/L = nanograms per liter, parts per trillion (ppt)		All units reported in ng/L	
RL= Reporting Limit		MDL=Method Detection Level	

8 DATA REDUCTION, VALIDATION, AND REPORTING

The following table presents data validation criteria for Field Reagent Blanks (FRB), specific to PFAS.

The FRB can be analyzed to assess whether PFAS in site samples could be non-site-related contamination and whether resampling is necessary, as discussed in Section 9. The FRB is only analyzed if the field sample PFAS target analyte is greater than the Reporting Limit (RL). If target analyte(s) detected in a field sample have an FRB concentration >1/3 the RL, flag the data and review with the project team to assess whether all samples should be recollected and reanalyzed.

9 INTERNAL QUALITY CONTROL

The 2000 QAPP requires that all target compounds be included in the Laboratory Control Sample (LCS) and Matrix Spike/Matrix Spike Duplicate (MS/MSD) analyses. This means that PFAS must be included in these QC samples. Definitions of LCS and MS/MSD can be found in the 2000 QAPP. All other measurement performance criteria (e.g., method blanks, surrogate recoveries, calibration criteria, etc.) are defined in the 2000 QAPP.

The following section applies to PFAS sampling and analysis.

9.1.5 FIELD REAGENT BLANK

A field reagent blank (FRB) will be included in the cooler within which the PFAS samples will be stored and shipped. The FRB can be analyzed to assess whether PFAS in site samples could be non-site-related contamination and whether resampling is necessary. The analysis of the sample is contingent upon detecting PFAS in sampled media above the Reporting Limit for that day's Sample Delivery Group (SDG).

The FRB is collected at the sampling site by:

1. Opening the bottle of chemically preserved FRB reagent water provided by the laboratory and a corresponding clean sample bottle also provided by the laboratory;
2. Pouring the preserved FRB reagent water into the empty sample bottle, closing the cap, labeling appropriately as the FRB and recording the sample on the chain-of-custodyform; and,
3. Pack and ship the FRB in the same cooler with the site samples to the laboratory (non-PFAS samples should not be included in the PFAS sample-containing cooler).

Table 9-2. PFAS Quality Control Check Summary

QC Checks	Frequency
Field Reagent Blank	One per day when PFAS is part of the Sample Delivery Group

FIGURE

Explanation

Grace Locations

- Monitoring Well Cluster
- Public Water Supply Well
- Former Recovery Well
- Recovery Well
- Decomissioned Recovery Well
- Non-Grace Recovery Well
- Former Reinjection Well

- Water Body
- Town Boundary
- Railroad
- Grace Property Boundary
- Proposed PFAS Sampling Location Phase 1
- Proposed PFAS Sampling Location Phase 2
- Contour (2018)

DRAFT

0 600 1,200 Feet

N

TITLE:	**Proposed PFAS Sampling Locations**
LOCATION:	W.R. Grace, Acton, Massachusetts
TETRA TECH	CHECKED: ED
	DRAFTED: JML
	DATE: 06/5/2019
	FIGURE: **1**

ATTACHMENT 1

LABORATORY STANDARD OPERATING PROCEDURE

Title: Per- and Polyfluorinated Substances (PFAS) in Water, Soils, Sediments and Tissue

[Method 537 (Modified), Method PFAS by LCMSMS Compliant with QSM 5.1 Table B-15]

Approvals (Signature/Date):

02/22/2019	02/25/2019
Robert Hrabak Date	Joe Schairer Date
Technical Manager	Health & Safety Manager / Coordinator
02/25/2019	02/26/2019
Lisa Stafford Date	Chris Williams Date
Quality Assurance Manager	Laboratory Manager

Facility Distribution No. Uncontrolled **Distributed To:** Sacramento Bids Folder

1. SCOPE AND APPLICATION

1.1. This procedure describes the analysis of water, soil, sediment, and tissue samples for the following compounds using liquid chromatography / tandem mass spectrometry (LC/MS/MS).

Compound Name	Abbreviation	CAS #
Perfluoroalkylcarboxylic acids (PFCAs)		
Perfluoro-n-butanoic acid	PFBA	375-22-4
Perfluoro-n-pentanoic acid	PFPeA	2706-90-3
Perfluoro-n-hexanoic acid	PFHxA	307-24-4
Perfluoro-n-heptanoic acid	PFHpA	375-85-9
Perfluoro-n-octanoic acid	PFOA	335-67-1
Perfluoro-n-nonanoic acid	PFNA	375-95-1
Perfluoro-n-decanoic acid	PFDA	335-76-2
Perfluoro-n-undecanoic acid	PFUdA (PFUnA)	2058-94-8
Perfluoro-n-dodecanoic acid	PFDoA	307-55-1
Perfluoro-n-tridecanoic acid	PFTrDA	72629-94-8
Perfluoro-n-tetradecanoic acid	PFTeDA (PFTA)	376-06-7
Perfluoro-n-hexadecanoic acid (non-routine analyte)	PFHxDA	67905-19-5
Perfluoro-n-octadecanoic acid (non-routine analyte)	PFODA	16517-11-6
Perfluorinated sulfonic acids (PFSAs)		
Perfluoro-1-butanesulfonic acid	PFBS	375-73-5
Perfluoro-1-pentanesulfonic acid	PFPeS	2706-91-1
Perfluoro-1-hexanesulfonic acid	PFHxS	355-46-4
Perfluoro-1-heptanesulfonic acid	PFHpS	375-92-8
Perfluoro-1-octanesulfonic acid	PFOS	1763-23-1
Perfluoro-nonanesulfonic acid	PFNS	8789-57-2
Perfluoro-1-decanesulfonic acid	PFDS	335-77-3
Perfluoro-1-dodecansulfonic acid	PFDoS	79780-39-5
Perfluorinated sulfonamides (FOSA)		
Perfluoro-1-octanesulfonamide	FOSA	754-91-6
Perfluorinated sulfonamidoacetic acids (FOSAA)		
N-ethylperfluoro-1-octanesulfonamidoacetic acid	EtFOSAA	2991-50-6
N-methylperfluoro-1-octanesulfonamidoacetic acid	MeFOSAA	2355-31-9
Fluorotelomer sulfonates (FTS)		
1H,1H,2H,2H-perfluorohexane sulfonate *(4:2)*	4:2 FTS	757124-72-4

Compound Name	Abbreviation	CAS #
1H,1H,2H,2H-perfluorooctane sulfonate *(6:2)*	6:2 FTS	27619-97-2
1H,1H,2H,2H-perfluorodecane sulfonate *(8:2)*	8:2 FTS	39108-34-4
1H,1H,2H,2H-perfluorododecane sulfonate *(10:2)*	10:2 FTS	120226-60-0

Abbreviations in parenthesis are the abbreviations listed in Method 537, where they differ from the abbreviation used by the laboratory's LIMS.

1.2. Additional analytes supported by this method: The following analytes can be supported by this method under special request.

Compound Name	Abbreviation	CAS #
Fluorinated Replacement Chemicals		
Dona (Donic acid)	Dona	919005-14-4
Perfluoro(2-propoxypropanoic) acid	HFPO-DA or GenX	13252-13-6
F53B (reported as the summation of the following)	F53B	NA
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonate	F53B major	73606-19-6
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonate	F5B minor	83329-89-9

1.3. The working range of the method is listed below. The linear range can be extended by diluting the extracts.

Matrix	Nominal Sample Size	Reporting Limit	Working Range
Water	250 mL	2.0 ng/L – 20 ng/L	2.0 ng/L - 400 ng/L
Soil/Sediment	5 g	0.2 ug/kg – 2.0 ug/kg	0.2 ug/kg - 40 ug/kg
Tissue	1 g	1.0 ug/kg – 10 ug/kg	1.0 ug/kg – 200 ug/kg

1.4. The procedure for the analysis of water samples via in line solid phase extraction (SPE) for a subset of the list in Section 1.1 using liquid chromatography / tandem mass spectrometry (LC/MS/MS) on a SCIEX 5500 is described in Attachment 1 of this SOP.

1.5. This procedure also includes direction for preparing and analyzing samples to determine "Total Oxidizable Precursors", which may assist in improving understanding of potential PFAS environmental risk.

1.6. When undertaking projects for the Department of Defense (DoD) and/or the Department of Energy (DOE) the relevant criteria in QA Policy WS-PQA-021, "Federal Program Requirements" must be checked and incorporated.

2. SUMMARY OF METHOD

2.1. Water samples are extracted using a solid phase extraction (SPE) cartridge. PFAS are eluted from the cartridge with an ammonium hydroxide/methanol solution.

2.2. Soil/sediment/tissue samples are extracted with a KOH/methanol solution using an orbital shaker for 3 hours followed by sonication for 12 hours. The mixture is centrifuged and the solvent filtered.

2.3. The final 80:20 methanol:water extracts are analyzed by LC/MS/MS. PFAS are separated from other components on a C18 column with a solvent gradient program using 20 mM ammonium acetate/water and methanol. The mass spectrometer detector is operated in the electrospray (ESI) negative ion mode for the analysis of PFAS.

2.4. An isotope dilution technique is employed with this method for the compounds of interest. The isotope dilution analytes (IDA) consist of carbon-13 labeled analogs, oxygen-18 labeled analogs, or deuterated analogs of the compounds of interest, and they are spiked into the samples at the time of extraction. This technique allows for the correction for analytical bias encountered when analyzing more chemically complex environmental samples. The isotopically labeled compounds are chemically similar to the compounds of concern and are therefore affected by sample-related interferences to the same extent as the compounds of concern. Compounds that do not have an identically labeled analog are quantitated by the IDA method using a closely related labeled analog.

2.5. Quantitation by the internal standard method is employed for the IDA analytes/recoveries. Peak response is measured as the area of the peak.

2.6. Samples for the "Total Oxidizable Precursor" assay (TOP) are analyzed in two phases – an aliquot is prepared and analyzed as a normal sample, and a second aliquot is subjected to oxidation with potassium persulfate and sodium hydroxide prior to solid phase extraction and analysis. The total perfluorocarboxylic acid value is determined for each aliquot, and the difference calculated.

3. DEFINITIONS

3.1. PFCAs: Perfluorocarboxylic acids

3.2. PFSAs: Perfluorinated sulfonic acids

3.3. FOSA: Perfluorinated sulfonamide

3.4. PFOA: Perfluorooctanoic acid

3.5. PFOS: Perfluorooctane sulfonic acid

3.6. PTFE: Polytetrafluoroethylene (e.g., Teflon®)

3.7. SPE: Solid phase extraction

3.8. PP: Polypropylene

3.9. PE: Polyethylene

3.10. HDPE: High density polyethylene

3.11. AFFF: Aqueous Film Forming Foam

3.12. IDA: Isotope dilution analyte

3.13. Further definitions of terms used in this SOP may be found in the glossary of the Laboratory Quality Assurance Manual (QAM).

4. INTERFERENCES

4.1. PFAS have been used in a wide variety of manufacturing processes, and laboratory supplies should be considered potentially contaminated until they have been tested and shown to be otherwise. The materials and supplies used during the method validation process have been tested and shown to be clean. These items are listed below in Section 6.

4.2. To avoid contamination of samples, standards are prepared in a ventilation hood in an area separate from where samples are extracted.

4.3. PTFE products can be a source of PFOA contamination. The use of PTFE in the procedure should be avoided or at least thoroughly tested before use. Polypropylene (PP) or polyethylene (PE, HDPE) products may be used in place of PTFE products to minimize PFOA contamination.

4.3.1. Standards and samples are injected from polypropylene autosampler vials with polypropylene screw caps once. Multiple injections may be performed on Primers when conditioning the instrument for analysis.

4.3.2. Random evaporation losses have been observed with the polypropylene caps causing high IDA recovery after the vial was punctured and sample re-injected. For this reason, it is best to inject standards and samples once in the analytical sequence.

4.3.3. Teflon-lined screw caps have detected PFAS at low concentrations. Repeated injection from the same teflon-lined screw cap have detected PFNA at increasing concentration as each repeated injection was performed, therefore, it is best to use polypropylene screw caps.

4.4. Volumetric glassware and syringes are difficult to clean after being used for solutions containing high levels of PFOA. These items should be labeled for use only with

similarly concentrated solutions or verified clean prior to re-use. To the extent possible, disposable labware is used.

4.5. Both branched and linear PFAS isomers can potentially be found in the environment. Linear and branched isomers are known to exist for PFOS, PFOA, PFHxS, PFBS, EtFOSAA, and MeFOSAA based upon the scientific literature. If multiple isomers are present for one of these PFAS they might be adjacent peaks that completely resolve or not, but usually with a deflection point resolved during peak integration. The later of these peaks matches the retention time of its labeled linear analog. In general, earlier peaks are the branched isomers and are not the result of peak splitting.

As of this writing, only PFOS, PFOA, and PFHxS are commercially available as technical mixtures. These reference standards of the technical mixtures for these specific PFAS are used to ensure that all appropriate peaks are included during peak integration.

4.6. In an attempt to reduce PFOS bias, it is required that *m/z* 499>80 transition be used as the quantitation transition.

4.7. Per the Certificate of Analysis for labeled perfluorohexadecanoic acid ($13C_2$-PFHxDA) produced by Wellington Laboratories, the stock standard contains roughly 0.3% of native perfluorohexadecanoic acid. This equates to roughly 0.30 ng/L or 0.015 ug/kg of perfluorohexadecanoic acid expected in all samples and blanks.

5. SAFETY

Employees must abide by the policies and procedures in the Corporate Safety Manual, Sacramento Supplement to the CSM, and this document. All work must be stopped in the event of a known or potential compromise to the health or safety of an associate. The situation must be reported **immediately** to a supervisor, the EH&S Staff, or a senior manager.

5.1. Specific Safety Concerns

5.1.1. Preliminary toxicity studies indicate that PFAS could have significant toxic effects. In the interest of keeping exposure levels as low as reasonably achievable, PFAS and PFAS samples must be handled in the laboratory as hazardous and toxic chemicals.

5.1.2. Exercise caution when using syringes with attached filter disc assemblies. Application of excessive force has, upon occasion, caused a filter disc to burst during the process.

5.1.3. Laboratory procedures such as repetitive use of pipets, repetitive transferring of extracts and manipulation of filled separatory funnels and other glassware represent a significant potential for repetitive motion or other ergonomic injuries. Laboratory associates performing these procedures are in the best

position to realize when they are at risk for these types of injuries. Whenever a situation is found in which an employee is performing the same repetitive motion, the employee shall immediately bring this to the attention of their supervisor, manager, or the EH&S staff. The task will be analyzed to determine a better means of accomplishing it.

5.1.4. Eye protection that satisfies ANSI Z87.1 (as per the TestAmerica Corporate Safety Manual), laboratory coat, and nitrile gloves must be worn while handling samples, standards, solvents, and reagents. Disposable gloves that have been contaminated will be removed and discarded; other gloves will be cleaned immediately.

5.1.5. Perfluorocarboxylic acids are acids and are not compatible with strong bases.

5.1.6. The use of vacuum systems presents the risk of imploding glassware. All glassware used during vacuum operations must be thoroughly inspected prior to each use. Glass that is chipped, scratched, cracked, rubbed, or marred in any manner must not be used under vacuum. It must be removed from service and replaced.

5.1.7. Glass containers are not to be used for "tumbling" soil samples.

5.2. Primary Materials Used

The following is a list of the materials used in this method, which have a serious or significant hazard rating. NOTE: This list does not include all materials used in the method. The table contains a summary of the primary hazards listed in the SDS for each of the materials listed in the table. A complete list of materials used in the method can be found in the reagents and materials section. Employees must review the information in the SDS for each material before using it for the first time or when there are major changes to the SDS.

Material[(1)]	**Hazards**	**Exposure Limit[(2)]**	**Signs and Symptoms of Exposure**
Acetic Acid (3-2-1)	Corrosive Poison Flammable	10 ppm-TWA 15 ppm-STEL	Contact with concentrated solution may cause serious damage to the skin and eyes. Inhalation of concentrated vapors may cause serious damage to the lining of the nose, throat, and lungs. Breathing difficulties may occur.

Material[1]	Hazards	Exposure Limit[2]	Signs and Symptoms of Exposure
Ammonium Hydroxide (3-0-0)	Corrosive Poison	50 ppm-TWA	Severe irritant. Effects from inhalation of dust or mist vary from mild irritation to serious damage to the upper respiratory tract. Symptoms may include sneezing, sore throat or runny nose. Contact with skin can cause irritation or severe burns and scarring with greater exposures. Causes irritation of eyes, and with greater exposures it can cause burns that may result in permanent damage, including blindness. Brief exposure to 5000 PPM can be fatal.
Hexane (2-3-0)	Flammable Irritant	500 ppm-TWA	Inhalation of vapors irritates the respiratory tract. Overexposure may cause lightheadedness, nausea, headache, and blurred vision. Vapors may cause irritation to the skin and eyes.
Hydrochloric Acid (3-0-1)	Corrosive Poison	5 ppm (Ceiling)	Can cause pain and severe burns upon inhalation, ingestion, eye or skin contact. Exposure to concentrated solutions may cause deep ulcerations to skin, permanent eye damage, circulatory failure and swallowing may be fatal.
Methanol (2-3-0)	Flammable Poison Irritant	200 ppm (TWA)	A slight irritant to the mucous membranes. Toxic effects exerted upon nervous system, particularly the optic nerve. Symptoms of overexposure may include headache, drowsiness and dizziness. Methyl alcohol is a defatting agent and may cause skin to become dry and cracked. Skin absorption can occur; symptoms may parallel inhalation exposure. Irritant to the eyes.
Potassium Hydroxide (3-0-1)	Corrosive Poison		Severe irritant. Can cause severe burns upon inhalation, ingestion, eye or skin contact. Exposure to concentrated solutions may cause severe scarring of tissue, blindness, and may be fatal if swallowed.
Potassium Persulfate (2-0-1-OX)	Oxidizer	None	Causes irritation to the respiratory tract. Symptoms may include coughing, shortness of breath. Causes irritation to skin and eyes. Symptoms include redness, itching, and pain. May cause dermatitis, burns, and moderate skin necrosis.
Sodium Hydroxide (3-0-1)	Corrosive Poison	2 mg/cm^3 (Ceiling)	Severe irritant. Can cause severe burns upon inhalation, ingestion, eye or skin contact. Exposure to concentrated solutions may cause severe scarring of tissue, blindness, and may be fatal if swallowed.

(1) Always add acid to water to prevent violent reactions.
(2) Exposure limit refers to the OSHA regulatory exposure limit.

6. EQUIPMENT AND SUPPLIES

6.1. 15 mL polypropylene test tubes with polypropylene screw caps.

6.2. 50 mL graduated plastic centrifuge tubes.

6.3. 125 mL HDPE bottles with HDPE screw caps.

6.4. 250 mL HDPE bottles with HDPE screw caps. The average weight of the HDPE bottles with HDPE screw caps are calibrated once per year. The calibration is performed by weighing 10 bottles with caps and dividing by 10 to get the average weight. The average weight is used in section (11.3.5.1.d).

6.5. Analytical balance capable of accurately weighing to the nearest 0.0001g, and checked for accuracy each day it is used in accordance with WS-QA-0041.

6.6. Extract concentrator or nitrogen manifold with water bath heating to 50-55°C.

6.7. Syringe filter, Millipore Millex-HV 0.45 um, or equivalent. Do not use PTFE type filters.

6.8. 300 µL autosampler vials, polypropylene, with polypropylene screw caps, Waters PN 1860004112, or equivalent.

6.9. SPE columns

6.9.1. Phenomenex Strata SPE C18, 6 mL, 500 mg, part number 8B-S002-HCH, Waters SepPak C18, 1 to 10g, or equivalent.

6.9.2. Waters Oasis WAX 150 mg/6 cc (PN 186002493) for the cleanup of solids.

6.9.3. Waters Oasis WAX 500 mg/6 cc (PN 186004647) for extraction of PFAS from aqueous sample.

6.9.4. Phenomenex Gemini 3 µm C18 110Å, 50 X 2 mm, Part No. 00B-4439-B0.

6.9.5. Phenomenex Luna 5 µm C18(2) 100Å, 30 X 3 mm, Part No. 00A-4252-Y0.

6.9.6. Penomenex Gemini 3 µm C18 110A, 50 X 3mm, Part No. 00B-4439-Y0.

6.10. Graphitized carbon (Envi-Carb™ or equivalent).

6.11. Vacuum manifold for Solid Phase Extraction (SPE).

6.12. Miscellaneous laboratory apparatus (beakers, test tubes, volumetric flasks, pipettes, etc.). These should be disposable where possible, or marked and segregated for high-level versus low-level use.

6.13. Water bath: Heated with concentric ring cover capable of temperature control (±5°C) up to 95°C. The bath must be used in a fume hood.

6.14. Plastic tub for an ice bath, AKRO-N.S.T. part No. 35-180 or equivalent.

6.15. pH indicator paper, wide range.

6.16. Bottle rotating apparatus for soil extractions.

6.17. Glass fiber filter, Whatman GF/F, catalog number 1825 090 or equivalent.

6.18. Liquid Chromatography/Tandem Mass Spectrometer (LC/MS/MS) – Either of the instruments described below, or equivalent, may be used for this method. Both HPLC are equipped with a refrigerated autosampler, an injection valve, and a pump capable of variable flow rate. The use of a column heater is required to maintain a stable temperature throughout the analytical run. Data is processed using Chrom Peak Review, version 2.1 or equivalent.

6.18.1. SCIEX LC/MS/MS

This system consists of a Shimadzu HPLC interfaced with a SCIEX 5500 Triple Quad MS. The instrument control and data acquisition software is SCIEX Analyst, version 1.6.3 or equivalent.

6.18.1.1. Shimadzu CTO-20AC HPLC equipped with 3 LC-20AD pumps and one DGU-20 degassing unit or equivalent.

6.18.1.2. Phenomenex Gemini C_{18} 3 um, 3.0 mm x 100 mm, Part No. 00D-4439-Y0, or equivalent.

6.18.1.3. PFAS Isolator column, Phenomenex Luna C_{18} 5 um, 50 mm x 4.6 mm, part no. 00B-4252-E0 or equivalent. This is plumbed between the UPLC pumps and autosampler valve to minimize PFAS background from the UPLC solvent lines and filters.

6.18.2. Waters LC/MS/MS

This consists of a Waters Acquity UPLC system interfaced with a Waters Quattro Premier tandem mass spectrometer. The instrument control and data acquisition software is MassLynx version 4.1, or equivalent.

6.18.2.1. Analytical column: Waters Acquity UPLC BEH C18 1.7 um, 3.0 mm x 150 mm, Part No. 186004690

6.18.2.2. PFAS Isolator column, Waters Acquity UPLC BEH Shield RP-18, 1.7 um, 2.1 mm x 50 mm, PN 186004476, or equivalent. This is plumbed between the UPLC pumps and autosampler valve to minimize PFAS background from the UPLC solvent lines and filters.

6.19. Preventive and routine maintenance is described in the table below

HPLC/MS/MS Preventative Maintenance	
As Needed: Change pump seals. Change in-line filters in autosampler (HPLC). Check/replace in-line frit if excessive pressure or poor performance. Replace column if no change following in-line frit change. Clean corona needle. Replace sample inlet tube in APCI (10.1 cm). Replace fused silica tube in ESI interface. Clean lenses. Clean skimmer. Ballast rough pump 30 minutes. Create all eluents in Reagent module, label eluent containers with TALS label and place 2nd label into maintenance log when put into use.	**Daily (When in use)** Check solvent reservoirs for sufficient level of solvent. Verify that pump is primed, operating pulse free. Check needle wash reservoir for sufficient solvent. Verify capillary heater temperature functioning. Verify vaporizer heater temperature. Verify rough pump oil levels. Verify turbo-pump functioning. Verify nitrogen pressure for auxiliary and sheath gasses. Verify that corona and multiplier are functioning.
Semi-Annually Replace rough-pump oil (4-6 months). Replace oil mist and odor elements. Replace activated alumina filter if applicable	**Annually** Vacuum system components including fans and fan covers. Clean/replace fan filters, if applicable.

7. REAGENTS AND STANDARDS

7.1. Reagent grade chemicals shall be used in all tests whenever available. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on the Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.1.1. Acetic acid, glacial

7.1.2. Ammonium acetate (20 mM in water): Prepared by weighing 1.509g of ammonium acetate and dissolving in 1L of water. The resultant solution is filtered through a 0.22um filter before use. This solution has volatile components, thus it should be replaced every 7 days or sooner.

7.1.3. Ammonium hydroxide (NH4OH), 0.3% in methanol: Prepared by diluting 12mL of ammonium hydroxide into 4L of methanol.

7.1.4. Hexane

7.1.5. Hydrochloric acid (HCl), 2.0 M solution in water

7.1.6. Hydrochloric acid (HCl), concentrated, reagent grade

7.1.7. Methanol

7.1.8. Potassium hydroxide (KOH), 0.4% in methanol: Prepared by weighing 16g of potassium hydroxide and dissolving in 4L of methanol.

7.1.9. Potassium persulfate, reagent grade

7.1.10. Ottawa Sand

7.1.11. Sodium hydroxide (NaOH), 0.1N, in water: Prepared by diluting 400mL of 1N NaOH into 3.6L of water for a total volume of 4L.

7.1.12. Sodium hydroxide (NaOH), 10N, reagent grade

7.1.13. Water, Nanopure or Millipore, must be free of interference and target analytes.

7.2. Standards

7.2.1. PFAS are purchased as high purity solids (96% or greater) or as certified solutions. Standard materials are verified compared to a second source material at the time of initial calibration. The solid stock material is stored at room temperature or as specified by the manufacturer or vendor.

7.2.1.1. Per the Certificate of Analysis for labeled perfluorohexadecanoic acid ($13C_2$-PFHxDA) produced by Wellington Laboratories, the stock standard contains roughly 0.3% of native perfluorohexadecanoic acid. This equates to roughly 0.30 ng/L or 0.015 ug/kg of perfluorohexadecanoic acid expected in all samples and blanks.

7.2.2. If solid material is used for preparing a standard, stock standard solutions are prepared from the solids and are stored at 4 ± 2°C. Stock standard solutions should be brought to room temperature before using. Standards are monitored for signs of degradation or evaporation. Standard solutions must be replaced at least annually from the date of preparation.

7.2.3. PFBS, PFHxS, PFHpS, PFOS, PFDS, and many other PFAS are not available in the acid form, but rather as their corresponding salts, such as sodium or potassium. The standards are prepared and corrected for their salt content according to the equation below.

$Mass_{acid}$ = Measured $Mass_{salt}$ × MW_{acid} / MW_{salt}

Where: MW_{acid} is the molecular weight of PFAA

MW_{salt} is the molecular weight of the purchased salt.

7.2.4. For example, the molecular weight of PFOS is 500.1295 and the molecular weight of NaPFOS is 523.1193. Therefore, the amount of NaPFOS used must be adjusted by a factor of 0.956.

7.3. Calibration Standards

The calibration stock solution is prepared by diluting the appropriate amounts of stock solutions in 80% methanol/water. The calibration stock solution is diluted with methanol to produce initial calibration standards. These are the normal calibration levels used. A different range can be used if needed to achieve lower reporting limits or a higher linear range.

7.4. Initial Calibration (ICAL) Levels (ng/mL)

Compound	CS-1	CS-2	CS-3	CS-4	CS-5	CS-6	CS-7
Perfluoroalkylcarboxylic acids (PFCAs)							
PFBA	0.5	1.0	5.0	20	50	200	400
PFPeA	0.5	1.0	5.0	20	50	200	400
PFHxA	0.5	1.0	5.0	20	50	200	400
PFHpA	0.5	1.0	5.0	20	50	200	400
PFOA	0.5	1.0	5.0	20	50	200	400
PFNA	0.5	1.0	5.0	20	50	200	400
PFDA	0.5	1.0	5.0	20	50	200	400
PFUdA	0.5	1.0	5.0	20	50	200	400
PFDoA	0.5	1.0	5.0	20	50	200	400
PFTrDA	0.5	1.0	5.0	20	50	200	400
PFTeDA	0.5	1.0	5.0	20	50	200	400
PFHxDA	0.5	1.0	5.0	20	50	200	400
PFODA	0.5	1.0	5.0	20	50	200	400
Perfluorinated sulfonic acids (PFSAs)							

Compound	CS-1	CS-2	CS-3	CS-4	CS-5	CS-6	CS-7
PFBS	0.5	1.0	5.0	20	50	200	400
PFPeS	0.5	1.0	5.0	20	50	200	400
PFHxS *	0.5	1.0	5.0	20	50	200	400
PFHpS	0.5	1.0	5.0	20	50	200	400
PFOS *	0.5	1.0	5.0	20	50	200	400
PFNS	0.5	1.0	5.0	20	50	200	400
PFDS	0.5	1.0	5.0	20	50	200	400
PFDoS	0.5	1.0	5.0	20	50	200	400
Perfluorinated sulfonamides (FOSA)							
FOSA	0.5	1.0	5.0	20	50	200	400
Perfluorinated sulfonamidoacetic acids (FOSAA)							
EtFOSAA	0.5	1.0	5.0	20	50	200	400
MeFOSAA	0.5	1.0	5.0	20	50	200	400
Fluorotelomer sulfonates (FTS)							
4:2 FTS	0.5	1.0	2.0	20	50	200	400
6:2 FTS	0.5	1.0	5.0	20	50	200	400
8:2 FTS	0.5	1.0	5.0	20	50	200	400
10:2 FTS	0.5	1.0	5.0	20	50	200	400
Labeled Isotope Dilution Analytes (IDA)							
13C4-PFBA	50	50	50	50	50	50	50
13C5-PFPeA	50	50	50	50	50	50	50
13C2-PFHxA	50	50	50	50	50	50	50
13C4-PFHpA	50	50	50	50	50	50	50
13C4-PFOA	50	50	50	50	50	50	50
13C5-PFNA	50	50	50	50	50	50	50
13C2-PFDA	50	50	50	50	50	50	50
13C2-PFUdA	50	50	50	50	50	50	50
13C2-PFDoA	50	50	50	50	50	50	50
18O2-PFHxS	50	50	50	50	50	50	50
13C4-PFOS	50	50	50	50	50	50	50
13C3-PFBS	50	50	50	50	50	50	50
13C2-PFTeDA	50	50	50	50	50	50	50
13C2-PFHxDA	50	50	50	50	50	50	50
13C8-FOSA	50	50	50	50	50	50	50
d5-EtFOSAA	50	50	50	50	50	50	50
d3-MeFOSAA	50	50	50	50	50	50	50
M2-4:2FTS ǂ	50	50	50	50	50	50	50
M2-6:2FTS	50	50	50	50	50	50	50
M2-8:2FTS	50	50	50	50	50	50	50
Internal Standard (IS)							

Compound	CS-1	CS-2	CS-3	CS-4	CS-5	CS-6	CS-7
13C2-PFOA	50	50	50	50	50	50	50

** Both branched and linear isomers are used.*

ǂ - This compound is used as a reverse surrogate for the TOP analysis.

***Note**: Sample extracts are in 80% MeOH/H $_2$O.*

Compound	CS-1	CS-2	CS-3	CS-4	CS-5	CS-6	CS-7
Fluorinated Replacement Chemicals							
HFPO-DA	0.5	1.0	5.0	20	50	200	400
9CI-PF3ONS (F53B major)	0.5	1.0	5.0	20	50	200	400
11CI-PF3OUdS (F53B minor)	0.5	1.0	5.0	20	50	200	400
Dona	0.5	1.0	5.0	20	50	200	400
Labeled Isotope Dilution Analytes							
13C3-HFPO-DA	0.5	1.0	5.0	20	50	200	400

***Note**: Sample extracts are in 80% MeOH/H $_2$O.*

***Note**: The above calibration limits are provided only as an example. The actual ICAL level used for each analytical batch will depend upon the LOQ requirements of the program. The concentration of the calibration solutions for non-concentrated extracts is 1/20th the levels indicated above.*

7.4.1. A technical (qualitative) grade PFOA standard which contains both linear and branched isomers is used as a retention time (RT) marker. This is used to integrate the total response for both linear and branched isomers of PFOA in environmental samples while relying on the initial calibration with the linear isomer quantitative standard This technical (qualitative) grade PFOA standard is analyzed initially, after every initial calibration or when significant changes are made to the HPLC parameters.

7.4.1.1. Attach this document to the ICV from the associated ICAL by scanning the document and associating it to the file as a document type of High Res MS Tune in TALS. Use the following naming convention: "_ZbatchnumberTPFOA".

7.5. Initial Calibration Verification Standard (ICV)

A second source solution for PFAS is purchased from the same vendor; the PFC-MXB contains most of the target analytes in this mixture and is used as an ICV. A few compounds are not available in this mixture, may not be available as another lot, and are not available from another vendor. For these analytes only, a second analyst may prepare a second source standard from the same source as the ICAL to produce an ICV. The recommended concentration of the ICV standard should be in the mid-range of the

calibration curve. The concentration may be adjusted if the initial calibration levels are changed or altered. The IDA and IS are added at a fixed concentration of 50 ng/mL.

7.6. LCS/Matrix PFC Spike Solution, 20 ng/mL

The PFC spike solution is prepared by diluting all PFAS to produce a solution containing each PFAS at a concentration of 20 ng/mL in methanol.

7.7. PFC Isotope Dilution Analyte Solution, 50 ng/mL

The PFC-IDA solution is prepared by diluting all labeled PFAS to produce a solution containing each compound at a concentration of 50 ng/mL in methanol.

7.8. Reverse Surrogate Solution, 1000 ng/mL

The reverse surrogate solution is prepared by diluting M2-4:2 FTS to produce a solution containing this compound at a concentration of 1000 ng/mL in methanol. This is added to all samples for the TOP assay to monitor the efficiency of the oxidation process.

7.9. Internal Standard Solution, 250 ng/mL

The internal standard solution is prepared by diluting 13C2-PFOA to produce a solution containing this compound at a concentration of 250 ng/mL in methanol. This is added to all extracts prior to analysis. The internal standard solution used for the non-concentrated extracts is at a concentration of 50 ng/mL.

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1. Water samples are collected in pre-cleaned 250 mL HDPE containers. Soil samples are collected in pre-cleaned 8 oz. HDPE containers. Other containers may also be suitable. Samples are chilled to 0 - 6°C for shipment to the laboratory.

8.1.1. Water samples collected from a known chlorinated source should be preserved with Trizma.

8.2. Samples are logged in following normal laboratory procedures and are stored under refrigeration at 0 - 6°C. Water samples must be extracted within 14 days of collection. Soil samples must also be extracted within 14 days of collection. Tissue samples must be extracted within 1 year of collection if stored at -20°C. Extracts must be refrigerated at 0 - 6°C, and analyzed within 40 days from extraction.

8.2.1. Projects performed for the state of New Jersey have an analytical holding time 28 days from the extraction date.

8.2.2. For projects performed for the state of New Jersey a field reagent blank (FRB) must be collected with each sample set. Acceptance limits are <RL for each analyte.

***Note**: As of this writing, Method 537 provides for a 14 day holding time for water samples preserved with Trizma buffer. The scientific literature indicates that perfluorinated substances are highly persistent in the environment. TestAmerica Sacramento has conducted time stability studies that support a 14 day holding time for aqueous samples with and without Trizma preservation. TestAmerica Denver has conducted stability studies indicating that medium- and low-level solutions of PFOA are stable for at least three months in polystyrene and polypropylene plastics at 0-6°C. The 14/40 day holding times given above are based on the stability study and general EPA convention for the holding time of extractable organic compounds in water and soil.*

9. QUALITY CONTROL

9.1. Initial Demonstration of Capability (IDOC)

The initial demonstration and method detection limit (MDL) studies described in Section 13 must be acceptable before analysis of samples may begin.

9.2. Batches are defined at the sample preparation step. Batches should be kept together through the whole analytical process as far as possible, but it is not mandatory to analyze prepared extracts on the same instrument or in the same sequence. Refer to the QC program document (WS-PQA-003) for further details of the batch definition.

9.2.1. The quality control batch is a set of up to 20 samples of the same matrix processed using the same procedure and reagents within the same time period. The quality control batch must contain a matrix spike/matrix spike duplicate (MS/MSD), a laboratory control sample (LCS) and a method blank. Laboratory generated QC samples (Blank, LCS, MS/MSD) do not count toward the maximum 20 samples in a batch. Field QC samples are included in the batch count. In some cases, at client request, the MS/MSD may be replaced with a matrix spike and sample duplicate. If insufficient sample is available for an MS/MSD, an LCSD may be substituted if batch precision is required by the program or client. In the event that multiple MS/MSDs are run with a batch due to client requirements, the additional MS/MSDs do not count toward the maximum 20 samples in a batch.

9.3. One method blank (MB, laboratory reagent blank) must be extracted with every process batch of similar matrix, not to exceed twenty (20) samples. For aqueous samples, the method blank is an aliquot of laboratory reagent water. For solid samples, the method blank is an aliquot of Ottawa sand. The method blank is processed in the same manner and at the same time as the associated samples. Corrective actions must be documented on a Non-Conformance memo, and then implemented when target analytes are detected in the method blank above the reporting limit or when IDA recoveries are outside of the control limits. Re-extraction of the blank, other batch QC and the affected samples are required when the method blank is deemed unacceptable. See policy WS-PQA-003 for specific acceptance criteria.

9.3.1. If the MB produces a peak within the retention time window of any of the analytes, determine the source of the contamination and eliminate the interference before processing samples.

9.3.2. The method blank must not contain any analyte at or above the reporting limit, or at or above 10% of the measured concentration of that analyte in the associated samples, whichever is higher.

9.3.3. If there is no target analyte greater than the RL in the samples associated with an unacceptable method blank, the data may be reported with qualifiers. Such action should be taken in consultation with the client.

9.3.4. Re-extraction and reanalysis of samples associated with an unacceptable method blank is required when reportable concentrations are determined in the samples.

9.3.5. Refer to WS-PQA-003 for further details of the corrective actions.

9.3.6. Projects performed under the auspices of the DOD/DOE must meet QSM specific criteria for method blanks. Results are acceptable if the blank contamination is less than ½ of the reporting limit/LOQ for each analyte, or less than 1/10 of the regulatory limit, or less than 1/10 of the sample result for the same analyte, whichever is greater. If the method blank does not meet the acceptance criteria, the source of contamination must be investigated and measures taken to correct, minimize or eliminate the problem. Reprepare and reanalyze all field and QC samples associated with the contaminated method blank.

9.4. A laboratory control sample (LCS) must be extracted with every process batch of similar matrix, not to exceed twenty (20) samples. The LCS is an aliquot of laboratory matrix (e.g. water for aqueous samples and Ottawa sand for solids) spiked with analytes of known identity and concentration. The LCS must be processed in the same manner and at the same time as the associated samples. Corrective actions must be documented on a Non-Conformance memo, then implemented when recoveries of any spiked analyte is outside of the control limits. Re-extraction of the blank, other batch QC, and all associated samples are required if the LCS is deemed unacceptable. See WS-PQA-0003 for specific acceptance criteria. The control limits for the LCS are stored in TALS.

9.4.1. Projects performed for the state of New Jersey: LCS (mid and high spike) recovery limits are 70-130%. Low level LCS recovery limits are 50-150%. The spike level must rotate between low, medium and high.

9.5. A matrix spike/matrix spike duplicate (MS/MSD or MS/SD) pair must be extracted with every process batch of similar matrix, not to exceed twenty (20) samples. An

MS/MSD pair is aliquots of a selected field sample spiked with analytes of known identity and concentration. The MS/MSD pair must be processed in the same manner and at the same time as the associated samples. Spiked analytes with recoveries or precision outside of the control limits must be within the control limits in the LCS. Corrective actions must be documented on a nonconformance memo, and then implemented when recoveries of any spiked analyte are outside of the control limits provided by TALS or by the client.

9.5.1. Projects performed for the state of New Jersey: MS/MSD (mid and high spike) recovery limits are 70-130%. Low level MS/MSD recovery limits are 50-150%. The spike level must rotate between low, medium and high.

9.6. A duplicate control sample (LCSD or DCS) may be added when insufficient sample volume is provided to process an MS/MSD pair, or is requested by the client. The LCSD is evaluated in the same manner as the LCS. See WS-PQA-003 for specific acceptance criteria.

9.7. Initial calibration verification (ICV) –A second source standard is analyzed with the initial calibration curve. The concentration should be at the mid range of the curve.

Corrective actions for the ICV include:

- Rerun the ICV.
- Remake or acquire a new ICV.
- Evaluate the instrument conditions.
- Evaluate the initial calibration standards.
- Rerun the initial calibration.

9.8. Isotope Dilution Analytes

9.8.1. The IDA solution is added to each field and QC sample at the time of extraction, as described in Section 11. As described in Section 7, this solution consists of isotopically labeled analogs of the analytes of interest.

9.8.2. IDA recoveries are flagged if they are outside of the acceptance limits (25–150%). Quantitation by isotope dilution generally precludes any adverse effect on data quality due to IDA recoveries being outside of the acceptance limits as long as the signal-to-nose ratio is greater than 10:1.

9.8.2.1. Evaluate data quality for usability, flag and submit a non-conformance memo for any analytes outside of the recovery criteria, and report if data is deemed not adversely effected.

9.8.2.2. Re-extraction of samples should be performed if the signal-to-noise for any IDA is less than 10:1 or if the IDA recoveries fall below 10%.

9.8.2.2.1. Re-extraction may be necessary under other circumstances when data quality has been determined to be adversely affected.

9.8.2.3. Projects performed under the auspices of the DoD/DOE must meet QSM 5.1 specific criteria for IDA recoveries which are 50-150%. If QC or field samples do not meet these criteria then re-extraction is required.

9.9. Internal Standard

9.9.1. The Internal Standard (IS) is added to each field and QC samples prior to analysis. The CCV IS response (peak area) must not deviate by more than 50% from the average response (peak area) of the initial calibration.

9.9.2. Sample IS response (peak area) must be within ±50% of the response (peak area) in the most recent CCV.

9.9.3. If the IS does not meet criteria, re-analyze the extract. If the IS meets criteria in the second analysis, report that analysis. If the IS does not meet criteria in the second analysis, report the first analysis with narration.

9.10. TOP Oxidation Efficiency

9.10.1. If the data indicates incomplete oxidation (i.e. the Post-TOP M2-4:2 FTS recovery is greater than 10% or the Post-TOP precursor concentration is greater than 10% of the Pre-TOP concentration) then a second aliquot (10 mL or a 0.2g equivalent) should be processed.

9.10.2. A reduced sample size may be used initially if sample history or other information indicates the sample is grossly contaminated.

9.11. Ion Ratio

9.11.1. Compare the quantifier/qualifier SRM transition ratio in the sample to the SRM transition ratio in the standard.

9.11.2. The quantifier/qualifier SRM ion ratio should be within + 50% of the average of the quantifier/qualifier SRM ion ratios calculated from the midlevel ICAL point or from the CCV, if an ICAL is not run.

9.11.3. At this time the ion ratio evaluation is a quantitative identification tool.

Analyst judgement should be used if the ratio does not meet criteria. Data should be qualified "I" if the ratio is not met.

10. CALIBRATION

10.1. For details of the calculations used to generate the regression equations, and how to use the factors generated by these equations, refer to SOP CA-Q-P-003 "Calibration Curves and Selection of Calibration Points".

10.2. Routine instrument operating conditions are listed in the table in Section 11.18.

10.3. Instrument Tuning

Instrument tuning is done initially when the method is first developed and thereafter as needed to maintain the sensitivity and selectivity of the method. Tuning is done by infusing each individual compound (native and IDA) into the mobile phase using a tee fitting at a point just before the entrance to the electrospray probe. The responses for the parent and daughter ions for each compound are observed and optimized for sensitivity and resolution. Mass assignments are reviewed and calibrated if necessary. The mass assignments must be within ± 0.5 amu of the values shown in the table in Section 11.18.

10.3.1. Once the optimal mass assignments (within ±0.5 amu of true) are made immediately following the initial tune, the lowest level standard from the initial calibration curve is assessed to ensure that a signal to noise ratio greater than 10 to 1 (S/N > 10:1) is achieved for each PFAS analyte. The first level standard from the initial calibration curve is used to evaluate the tune stability on an ongoing basis. The instrument mass windows are set initially at ± 0.5 amu of the true value; therefore, continued detection of the analyte transition with S/N > 10:1 serves as verification that the assigned mass remains within ± 0.5 amu of the true value, which meets the DoD/DOE QSM tune criterion. For QSM work, the instrument sensitivity check (section 10.12.4) is also evaluated to ensure that the signal to noise criteria is met.

10.4. A new calibration curve must be generated after major changes to the system or when the continuing calibration criteria cannot be met. Major changes include, but are not limited to, new columns or pump seals. A new calibration is not required after minor maintenance.

10.5. With the exception of the circumstances delineated in policy CA-Q-P-003, it is not acceptable to remove points from a calibration curve. In any event, at least five points must be included in the calibration curve. Average Response Factor and linear fit calibrations require five points, whereas Quadratic (second order) calibrations require six points.

10.6. A fixed injection volume is used for quantitation purposes and is to be the same for both the sample and standards.

10.7. All units used in the calculations must be consistently uniform, such as concentration in ng/mL.

10.8. Initial Calibration

10.8.1. A number of analytical standards of different analyte concentrations are used to generate the curve. Each standard is injected once to obtain the peak response for each analyte at each concentration. These standards define the working range of the analysis.

10.8.1.1. A minimum of five analytical standards is used when using average response factor and/or linear calibration fits.

10.8.1.2. A minimum of six analytical standards is used when a quadratic fit is used to generate the curve.

10.8.2. Calibration is by average response factor, linear fit, or by quadratic fit. Quadratic fit is used for the analyte if the response is non-linear.

10.8.2.1. For average response factor (RFa), the relative standard deviation (RSD) for all compounds quantitated against an identically labeled analog must be < 35% for the curve to be valid.

10.8.2.2. For average response factor (RFa), the relative standard deviation (RSD) for all compounds quantitated against a closely related labeled analog IDA must be < 50% for the curve to be valid.

10.8.2.3. For linear fit, the intercept of the line must be less than ½ the reporting limit, and the coefficient of determination (r2) must be greater than or equal to 0.990 for the curve to be considered valid (or the correlation coefficient (r) > 0.995).

10.8.2.4. The Internal Standard (IS) response (peak area) must not deviate by more than 50% from the average response (peak area) of the initial calibration.

10.8.2.5. Projects performed under the auspices of the DoD/DOE must meet QSM 5.1 specific criteria for initial calibration: The %RSD of the RFS for all analytes must be <20%. Linear or non-linear calibrations must have r^2>0.99 for each analyte. Analytes must be within 70-130% of their true value for each calibration standard.

10.8.2.6. Projects performed for the state of New Jersey: Each calibration point, except the lowest point, of each analyte should be calculated to be within 70-130% of the true value. The lowest calibration point that is at or below the MRL should be within 50-150% of its true value.

10.9. Calibration Curve Fits

10.9.1. Linear regression or quadratic curves may be used to fit the data to a calibration function. Detailed descriptions and formulas for each fitting type can be found in SOP CA-Q-P-003, "Calibration Curves and Selection of Calibration Points".

10.9.2. The linear curve uses the following function:

Equation 1

$$y = bx + c$$

Where:

$$y = \frac{\text{Area (analyte)}}{\text{Area (IS)}} \times \text{Concentration (IS)}$$

x = concentration
b = slope
c = intercept

10.9.3. The quadratic curve uses the following function:

Equation 2

$$y = ax^2 + bx + c$$

Where y, x, b, and c are the same as above, and a = curvature.

10.9.4. Evaluation of Calibration Curves

The following requirements must be met for any calibration to be used:

- Response must increase with increasing concentration.
- The absolute value of the intercept of a regression line (linear or non-linear) at zero response must be less than the reporting limit.
- There should be no carryover at or above 1/2 MRL after a high CAL standard.

If these criteria are not met, instrument conditions and standards will be checked, and the ICAL successfully repeated before continuing.

10.9.5. Weighting of Calibration Points

In linear and quadratic calibration fits, the points at the lower end of the calibration curve have less absolute variance than points at the high concentration end of the curve. This can cause severe errors in quantitation at the low end of the calibration. Because accuracy at the low end of the

curve is very important for this analysis, it is preferable to increase the weighting of the lower concentration points. 1/concentration or 1/x weighting is encouraged. Visual inspection of the line fitted to the data is important in selecting the best fit.

10.10. Initial Calibration Blank (ICB)

10.10.1. Immediately following the ICAL, a calibration blank is analyzed that consists of an injection of 80:20 methanol:water blank containing both IDA and IS.

10.10.2. The result for the calibration blank must be less than the reporting limit.

10.10.3. If the ICB is greater than the reporting limit then the source of contamination must be identified and any necessary cleaning completed, and then the instrument should be recalibrated.

10.10.4. Projects performed under the auspices of the DoD/DOE must meet QSM 5.1 specific criteria for instrument blanks. One is required immediately following the highest standard analyzed and *daily prior to sample analysis*. The instrument blank must be < ½ the LOQ.

10.11. Initial Calibration Verification (ICV)

10.11.1. Following the ICAL and the ICB, an ICV standard obtained from a different source or vendor than the ICAL standards is analyzed. This ICV standard is a mid-range standard.

10.11.2. The recovery for the ICV must meet the appropriate following criteria:

10.11.2.1. The native analyte must be within or equal to 60-140% for all native analytes quantitated against an identically labeled analog IDA.

10.11.2.2. The native analyte must be within or equal to 50-150% for all native analytes quantitated against a closely related labeled analog IDA.

10.11.2.3. The IDA must be within or equal to 50-150%.

10.11.3. Projects performed under the auspices of the DoD/DOE QSM (Version 5.1) and the state of New Jersey must meet these criteria for the ICV: Analyte concentrations must be within ±30% of their true values for all analytes, IDA and target.

10.11.4. See Section 9.7 for corrective actions in the event that the ICV does not meet

the criteria above.

10.12. Continuing Calibration Verification (CCV)

Analyze a CCV at the beginning of a run, the end of a run, and after every 10 samples to determine if the calibration is still valid. The exception is after an acceptable curve and ICV are run 10 samples can be analyzed before a CCV is required. The CCVs are usually at the mid-level range of the curve and should vary throughout the run from low level (LOQ/RL) to mid level. The curve and ICV do not need to be run every day. To start an analytical run a CCV can be analyzed and if it meets acceptance criteria a run can be started. In addition, the low standard in the curve must be analyzed and must be within ± 50% of the expected value.

10.12.1. The recovery for the CCV standards must be equal to or within 60-140% for all natives quantitated against an identically labeled analog and equal to or within 50% to 150% for all natives quantitated against a closely related labeled analog. The recovery for the IDA must be within or equal to 50-150%.

10.12.2. The Internal Standard (IS) response (peak area) must be within ± 50% from the response (peak area) from the midpoint of the initial calibration.

10.12.2.1. Sample IS response (peak area) must be within ± 50% of the response (peak area) in the most recent CCV.

10.12.3. If this is not achieved, the instrument has drifted outside the calibration limits. The instrument must be recalibrated.

10.12.4. Projects performed under the auspices of the DoD/DOE must meet QSM 5.1 specific criteria for CCV. All analyte concentrations must be within ± 30% of their true value. Additionally, prior to analysis and at least once every 12 hours an instrument sensitivity check (ISC/CCVL) must be analyzed. The analyte concentrations must be at LOQ and the concentrations must be within ± 30% of their true value. This can be used as a CCV.

10.12.5. Projects performed for the state of New Jersey: All analyte concentrations in the CCV must be within + 30% of their true value. All analyte concentrations in the low level CCV must be within + 50% of their true value.

11. PROCEDURE

11.1. One-time procedural variations are allowed only if deemed necessary in the professional judgment of a supervisor to accommodate variation in sample matrix, chemistry, sample size, or other parameters. Any variation in procedure shall be completely documented using an Non-Conformance Memo (NCM). The NCM process

is described in more detail in SOP WS-QA-0023. The NCM shall be filed in the project file and addressed in the case narrative.

Any deviations from this procedure identified after the work has been completed must be documented in an NCM, with a cause and corrective action described.

11.2. Water Sample Preparation

11.2.1. Visually inspect samples for the presence of settled and/or suspended sediment/particulates. If present or if the sample is biphasic add IDA prior to any sample decanting or centrifugation. If the sample requires decanting or centrifugation contact the client for guidance prior to such action. Decanting or filtering of the sample can lead to a low bias.

11.2.2. If authorized by the client to filter the sample, filter the water sample through a glass fiber filter (Whatman GF/F Cat No 1825 090 or equivalent). Gravity or vacuum can be used to pass the sample through the filter. Prepare a filtration blank with any samples requiring filtration. File an NCM noting the need for filtration.

Warning: The use of a vacuum system creates the risk of glassware implosion. Inspect all glassware prior to use. Glassware with chips, scratches, rub marks or cracks must not be used.

11.2.3. Weigh the sample container prior to extraction and then weigh the sample container after extraction to determine the initial volume. Unless otherwise directed by client, use the entire sample volume.

11.2.4. Prepare additional aliquots of a field sample for the MS/MSD, if requested.

11.2.5. Prepare two 250 mL aliquots of HPLC-grade water for the method blank and LCS.

11.2.6. Spike the LCS and MS/MSD (if requested) with 0.5 mL of the LCS/Matrix PFC Spike solution (Section 7.6). This will result in a sample concentration of 40 ng/L.

11.2.7. Add 0.5 mL of the IDA PFC solution (Section 7.7) into each sample and QC sample, for a fixed concentration of 50 ng/mL in the final sample vial.

11.3. Solid Phase Extraction (SPE) of Aqueous Samples

The automated Zymark Auto-Trace Workstation can be used as long as the program follows these conditions and passes the background check.

11.3.1. Condition the SPE cartridges (Waters WAX, 500 mg/6 cc) by passing the following without drying the column.

Note: *The cartridges should not be allowed to go dry until the final elution step with methanol. At all of the other transition steps, the solvent/sample level should be stopped at the top of the column before the next liquid is added.*

WARNING: The use of a vacuum system creates the risk of glassware implosion. Inspect all glassware prior to use. Glassware with chips, scratches, rub marks or cracks must not be used.

11.3.2. Wash with 5.0 mL of 0.3% NH_4OH/methanol.

11.3.3. Wash with 5.0 mL of 0.1N NaOH/water. Close valve when ~ 200 uL remains on top to keep column wet. After this step, the columns cannot go dry until the completion of loading and rinsing samples.

11.3.4. Appropriately label the columns and add the reservoir to the column.

11.3.5. Add samples to the columns and with vacuum, pull the entire 250 mL aliquot of the sample through the cartridge at a rate of approximately 2 to 5 drops per second.

11.3.5.1. If the SPE column should plug (flow rate <1 drop per minute) prior to the entire content of the sample container passing through the column do the following:

1. Stop adding sample to the reservoir.
2. Return any remaining sample volume back to the original container.
3. Weigh the original container and record this weight into the worksheet notes field within the TALS extraction batch.
4. Determine the full volume of sample fortified by using the "Gross Weight" – (remaining sample volume – default tare weight of a sample container (26.1 g)).
5. Enter this value into the "Initial Amount" field in the TALS extraction batch.
6. Proceed to Section 11.4, noting that additional vacuum or pressure might be needed to elute the SPE column.

11.3.6. After the entire sample has been loaded onto the column, rinse the sample bottle with two 5 mL aliquots of reagent water and pour onto the column reservoir.

11.3.7. After the final loading of the sample but before completely passed through the column, rinse the SPE column with 1 mL of water.

11.3.8. After the sample and water rinse have completely passed through the cartridge, allow the column to dry well with vacuum for 15 minutes.

11.4. SPE Column Wash of Aqueous Samples with Hexane

11.4.1. Load the first 5 mL of hexane to soak for five minutes and then elute to waste.

11.4.2. Load the second 5 mL of hexane and elute to waste (without a soaking period).

11.4.3. Allow the column to dry with vacuum for 5 to 10 minutes. Columns must be dried before continuing.

11.5. SPE Elution of Aqueous Samples – using 15 mL polypropylene test tubes as receiving tubes in the SPE manifold.

11.5.1. Rinse sample bottles with 5 mL of 0.3% NH_4OH/methanol and transfer to the column reservoir onto the cartridge. Allow the solution to soak for 5 minutes and then elute into the 15 mL collection tube.

11.5.2. Repeat sample bottle to column reservoir rinse and cartridge elution with a second 5 mL aliquot of 0.3% NH_4OH/methanol. The total collection should be approximately 10 mL.

11.5.3. **Note: If the extracts will not be concentrated elute extract with a total of 8 mL of 0.3% NH_4OH/methanol.**

11.5.4. Proceed to Section 11.15.2 (Graphitized Carbon Cleanup) as needed. This required for all DoD/DOE extracts.

11.6. Extract Concentration for Aqueous Extracts (Note, if the extract will not be concentrated, proceed to Section 11.7.)

11.6.1. Prior to concentrating each sample, add 100 uL of water.

11.6.2. Concentrate each sample under a gentle stream of nitrogen until the methanol is evaporated and the 100 uL of water remains.

11.6.2.1. This blow down must take a minimum of 3.5 hours.

11.6.2.2. Extracts can not remain in the water bath longer than 5 minutes once concentrated.

11.6.3. Add 300 uL of methanol and mix the contents well using a vortex mixer.

11.6.4. Add 100 uL of Internal Standard (IS) 250 ng/mL concentration solution to each extract and vortex to mix.

11.6.5. This will create an extract with a final solvent composition of 80:20 methanol:water.

11.6.6. Transfer a portion of the extract to a 300 uL polypropylene autosampler vial (7 drop-wise or approximately ½ filled is sufficient). Archive the rest of the extracts for re-injection and dilution.

11.6.7. Seal the vial with a polypropylene screw cap. Note: Teflon lined caps can not be used due to detection of low level concentration of PFAS.

11.7. Final volume for non-concentrated extract

11.7.1. If the extract does not undergo concentration add 0.5 mL of IS 50 ng/mL concentration and 2 mL of water to the extract. This will create an extract with a final solvent composition of 80:20 methanol:water.

11.7.1.1. Seal the test tube tightly. Invert container several times and then vortex. Allow extract to settle for 10 minutes prior to moving to the next step.

11.7.2. Transfer a portion of the extract to a 300 uL polypropylene autosampler vial (7 drop-wise or approximately ½ filled is sufficient). Archive the rest of the extracts for re-injection and dilution.

11.7.3. Seal the vial with a polypropylene screw cap. Note: Teflon lined caps cannot be used due to detection of low level concentration of PFAS.

11.8. Soil, Sediment and Tissue Sample Preparation and Extraction

11.8.1. Visually inspect soil samples for homogeneity.

11.8.1.1. Projects performed under the auspices of the DoD/DOE must have the entire sample homogenized prior to subsampling in accordance with QSM 5.1 criteria (see SOP WS-QA-0018).

11.8.2. Weigh a representative 5 g aliquot of soil, sediment or 1 g of tissue sample into a 50 mL HDPE wide-mouth bottle. Weigh additional sample amounts for the matrix spike and matrix spike duplicate analyses if they are requested.

11.8.3. For the method blank and LCS matrix, use 5 g each of Ottawa sand or 0.1 g of oil.

11.8.4. Spike the LCS and MS/MSD (if requested) with 1.0 mL of the LCS/Matrix PFC Spike solution (Section 7.6). This will result in a sample concentration of 4.0 ng/g.

11.8.4.1. Spike non-concentrated samples at 0.5 mL of LCS/Matrix PFC Spike Solution.

11.8.5. Add 1.0 mL of the IDA PFC solution (Section 7.7) into each sample and QC sample, for a fixed concentration of 50 ng/mL in the final sample vial.

11.8.5.1. Spike non-concentrated samples at 0.5 mL of IDA PFC Solution.

11.8.6. Cap the bottles and allow the spike to settle into the sample matrix. Gently shake the bottles to mix the spike into the matrix.

11.8.7. Add 20 mL of 0.4% KOH/methanol to each sample.

11.8.8. Shake each sample on an orbital shaker at room temperature for 3 hours.

11.8.9. Following the shaking, extract the samples in an ultrasonic water bath for an additional 12 hours.

11.8.10. After the completion of extraction, centrifuge each sample at 3500 rpm for 15 minutes.

11.8.11. Collect and decant the KOH/methanol extract to a new 50 mL centrifuge tube.

11.8.12. Add another 2 mL of 0.4% KOH/methanol solution to the residue, briefly shake to mix and centrifuge at 3500 rpm for 15 minutes.

11.8.13. Combine the rinsate to the first corresponding tubes.

11.8.14. To the final KOH/methanol extract, add 2 mL of water to each.

11.8.15. Concentrate the KOH/methanol/water extract under nitrogen to less than 2 mL, and dilute with water to 15 mL final volume.

11.8.16. Acidify with 80 uL of glacial acetic acid, and mix the contents well with vortex mixer. Check the pH to ensure pH is between 6 to 8.

11.8.17. Centrifuge at 3500 rpm for 15 minutes.

11.9. Solid Extract Cleanup by SPE

Set up WAX 150 mg/6 cc SPE columns for sample cleanup using vacuum manifold.

11.9.1. Condition the SPE cartridges by passing the following without drying the column.

Note: *The cartridges should not be allowed to go dry until the final elution step with methanol. At all of the other transition steps, the solvent/sample level should be stopped at the top of the column before the next liquid is added.*

WARNING: The use of a vacuum system creates the risk of glassware implosion. Inspect all glassware prior to use. Glassware with chips, scratches, rub marks or cracks must not be used.

11.9.2. Wash with 5.0 mL of 0.3% NH_4OH/methanol.

11.9.3. Wash with 10 mL of 0.1N NaOH/water. Close valve when ~ 500uL remains on top of column to keep column wet. *After this step, the columns cannot go dry until the completion of loading and rinsing samples.*

11.9.4. Add extracts to the columns and with vacuum, pull the entire extracts through the cartridge at rate of approximately 3 to 5 drops per second.

11.9.5. Rinse the sample tube with 5 mL of water and add to the SPE column.

11.9.6. Dry the columns with vacuum for 15 minutes.

11.10. SPE Column Wash of Solid Extracts with Hexane

11.10.1. Load the first 5 mL of hexane to soak for five minutes, and elute to waste.

11.10.2. Load the second 5 mL of hexane and elute to waste (without a soaking period).

11.10.3. Allow the column to dry with vacuum for 10 minutes. Columns must be dried before continuing.

11.11. SPE Elution of Solid Extracts – using 15 mL polypropylene test tube as receiving tube in the SPE manifold.

11.11.1. Rinse extraction bottles with 5 mL of 0.3% NH_4OH/methanol and transfer to the column reservoir onto the cartridge. Allow the solution to soak for 5 minutes and then elute into the 15 mL collection tube.

11.11.2. Repeat extract bottle to column reservoir rinse and cartridge elution with a second 5 mL aliquot of 0.3% NH_4OH/methanol. The total collection should be approximately 10 mL.

11.11.3. **Note: If the extracts will not be concentrated elute extract with a total of 8 mL of 0.3% NH_4OH/methanol.**

11.11.4. Proceed to Section 11.15.2 (Graphitized Carbon Cleanup) as needed. This is

required for all DoD/DOE extracts.

11.12. Extract Concentration for Solid Samples (Note, if the extract will not be concentrated, proceed to Section 11.7)

11.12.1. Prior to concentrating each sample, add 200 uL of water.

11.12.2. Concentrate each sample under a gentle stream of nitrogen until the methanol is evaporated and the 200 uL of water remains.

11.12.2.1. This blow down must take a minimum of 3.5 hours.

11.12.2.2. Extracts can not remain in the water bath longer than 5 minutes once concentrated.

11.12.2.3. Add 600 uL of methanol and mix the contents well using a vortex mixer.

11.12.2.4. Add 200 uL of Internal Standard (IS) 250 ng/mL concentration solution to each extract and vortex to mix.

11.12.3. Transfer a portion of the extract to a 300 uL polypropylene autosampler vial (7 drop-wise or approximately ½ filled is sufficient). Archive the rest of the extracts for re-injection and dilution.

11.12.4. Seal the vial with a polypropylene screw cap. *Note: Teflon lined caps can not be used due to detection of low level concentration of PFAS.*

11.13. Product/Dispersion Samples

11.13.1. Check the solubility of the material in both methanol and water

11.13.1.1. If the material is soluble in water, dilute 0.5 mL of sample into 250 mL of DI water and proceed to Section 11.3 (follow water extraction procedures). Fortify sample appropriately with IDA or PFC spike solution, see Section 11.2.

11.13.1.2. If the material is soluble in methanol, dilute 1 g (if solid) or 1 mL (if liquid) of material into 10 mL of methanol (MeOH).

11.13.1.2.1. If the material does not completely dissolve, contact your immediate supervisor.

11.13.2. Take 100 uL of the 10 mL solution and dilute it to 10 mL in MeOH.

11.13.3. Take a 1 mL aliquot of this solution (effective dilution of 1000x (1 mg for solid or 0.001 mL for liquid)) and fortify with 0.5 mL of labeled IDA

solution (Section 7.7).

11.13.4. DO NOT PASS EXTRACT THROUGH SPE CARTIRIDGE (omit steps 11.9 – 11.11).

11.13.5. Proceed to Section 11.6 of this SOP for extract concentration.

11.14. TOP (Total Oxidizable Precursor) Assay for Aqueous Samples

11.14.1. Prepare 3-250 mL HDPE containers with HPLC grade water to create the needed QC Samples (MB, LCS/LCSD).

11.14.2. Prepare enough 125 mL HDPE containers as needed for all "Pre" and "Post" samples, including QC. Label each appropriately.

11.14.3. Spike the "Pre" and "Post" MB 125 mL containers with 25 uL of the reverse surrogate solution of M2-4:2 FTS (Section 7.8).

11.14.4. Spike the "Pre" and "Post" LCS/LCSD 125 mL containers with 0.5 mL of the LCS Spike solution (Section 7.6), both regular and "add-on", and 25 uL of the reverse surrogate solution (Section 7.8).

11.14.5. Remove the methanol solvent from all Post QC sample 125 mL containers (MB and LCS/LCSD) by using N2 evaporation.

11.14.6. Add 2g of potassium persulfate and 1.9 mL of 10 M NaOH to each "Post" sample container.

11.14.7. Subsample 100 mL aliquots of water from each field sample and QC from the 250 mL containers into each of the corresponding 125 mL containers for both the "Pre" and "Post" samples. Spike all "Pre" and "Post" samples with 25uL of the reverse surrogate solution (Section 7.8).

11.14.8. Set aside all "Pre" sample containers.

11.14.9. Cap each "Post" sample container, invert 2-3 times prior to placing container into water bath.

11.14.10. Add 2 g of potassium persulfate and 1.9 mL of 10N NaOH to each "Post" sample container.

11.14.11. Heat each "Post" sample container in a water bath (KD) at 85°C for 6 hours.

11.14.12. After digestion for 6 hours, place the "Post" sample containers in an ice bath for 30 minutes.

11.14.13. Adjust the pH of "Post" samples and associated QC aliquots to 7 with concentrated HCl. Use pH paper to determine the pH.

11.14.14. Spike both "Pre" and "Post" samples and their associated QC samples with 0.5 mL of PFC IDA solution (Section 7.7), both regular and add-on.

11.14.15. Use the following SPE procedure for both "Pre" and "Post" samples:

11.14.15.1. Set up WAX 150 mg/6 cc SPE columns for sample extraction using a vacuum manifold.

11.14.15.2. Establish a sample loading flow rate of 3-5 drops per second for each port of the vacuum manifold, for as many ports as will be used simultaneously during sample loading.

11.14.15.3. Wash/condition the SPE column with 5 mL of 0.3% NH_4OH/Methanol, then 5 mL water.

11.14.15.4. Load 100 mL of sample onto the SPE cartridge at a flow rate of 3-5 drops per second.

11.14.15.5. Add 5 mL rinse water

11.14.15.6. After the sample and water rinse have completely passed through the column, allow it to dry well using vacuum with a flow rate of 1 mL/minute for 15 minutes.

11.14.15.7. Wash the SPE column with 10 mL hexane rinse eluting all to waste.

11.14.15.8. Allow the column to dry well using vacuum for 5 minutes. Columns must be dry before continuing.

11.14.15.9. Elute the samples into 15 mL polypropylene test tubes in the SPE manifold by rinsing each 125 mL sample container with 5 mL of 0.3% NH_4OH/methanol, and add to the SPE cartridge as eluent.

11.14.15.10. Repeat with another 5 mL of 0.3% NH_4OH/methanol.

11.14.15.11. Collect the 10 mL of eluent and concentrate per Section 11.6.

11.15. TOP (Total Oxidizable Precursor) Assay for Soil Samples

11.15.1. Weigh representative 2 g aliquots of soil for each "Pre" and "Post" sample into a 50 mL centrifuge tube.

11.15.2. For the method blank and LCS matrix, use 2 g each of Ottawa sand for each

"Pre" and "Post" QC sample.

11.15.3. Add 20 mL of 0.4% KOH/methanol to each sample.

11.15.4. Shake each sample on an orbital shaker at room temperature for 3 hours.

11.15.5. Following the shaking, extract the samples in an ultrasonic water bath for an additional 12 hours.

11.15.6. After the completion of extraction, centrifuge each sample at 3500 rpm for 15 minutes.

11.15.7. Collect and decant the KOH/methanol extract to a new 50 mL centrifuge tube.

11.15.8. Add another 2 mL of 0.4% KOH/methanol solution to the residue, briefly shake to mix and centrifuge at 3500 rpm for 15 minutes.

11.15.9. Combine the rinsate to the first corresponding tubes.

11.15.10. Proceed to Section 11.16.2 (Envi-carb clean up)

11.15.11. To the final KOH/methanol extract, add 0.5 mL of water to each.

11.15.12. Concentrate the KOH/methanol/water extract under nitrogen to less than 0.25 mL.

11.15.13. Dilute extract up to 50 mL with water in the centrifuge tube and vortex.

11.15.14. Prepare enough 125 mL HDPE containers as needed for all "Pre" and "Post" samples, including QC. Label each appropriately.

11.15.15. Spike the "Pre" and "Post" MB 125 mL containers with 25 uL of the reverse surrogate solution of M2-4:2 FTS (Section 7.8).

11.15.16. Spike the "Pre" and "Post" LCS/LCSD 125 mL containers with 0.5 mL of the LCS Spike solution and 25 uL of the reverse surrogate solution (Section 7.8).

11.15.17. Remove the methanol solvent from all "Post" QC sample 125 mL containers (MB and LCS/LCSD) by using N2 evaporation.

11.15.18. Add 2g of potassium persulfate and 1.9 mL of 10N NaOH to each "Post" sample container.

11.15.19. Transfer extract from the centrifuge tube to the appropriate 125 mL

container.

11.15.20. Rinse the centrifuge container with an additional 50 mL of water and transfer to the appropriate 125 mL container.

11.15.21. Set aside all "Pre" sample containers.

11.15.22. Cap each "Post" sample container, invert 2-3 times prior to placing container into water bath.

11.15.23. Heat each "Post" sample container in a water bath (KD) at 85°C for 6 hours.

11.15.24. After digestion for 6 hours, place the "Post" sample containers in an ice bath for 30 minutes.

11.15.25. Adjust the pH of "Post" samples and associated QC aliquots to 7 with concentrated HCl. Use pH paper to determine the pH.

11.15.26. Spike both "Pre" and "Post" samples and their associated QC samples with 0.5 mL of PFC IDA solution (Section 7.7).

11.15.27. Use the following SPE procedure for both "Pre" and "Post" samples:

11.15.27.1. Set up WAX 150 mg/6 cc SPE columns for sample extraction using a vacuum manifold.

11.15.27.2. Establish a sample loading flow rate of 3-5 drops per second for each port of the vacuum manifold, for as many ports as will be used simultaneously during sample loading.

11.15.27.3. Wash/condition the SPE column with 5 mL of 0.3% NH_4OH/Methanol, then 5 mL water.

11.15.27.4. Load 100 mL of sample onto the SPE cartridge at a flow rate of 3-5 drops per second.

11.15.27.5. Add 5 mL rinse water

11.15.27.6. After the sample and water rinse have completely passed through the column, allow it to dry well using vacuum with a flow rate of 1 mL/minute for 15 minutes.

11.15.27.7. Wash the SPE column with 10 mL hexane rinse eluting all to waste.

11.15.27.8. Allow the column to dry well using vacuum for 5 minutes. Columns must be dry before continuing.

11.15.27.9. Elute the samples into 15 mL polypropylene test tubes in the SPE manifold by rinsing each 125 mL sample container with 5 mL of 0.3% NH_4OH/methanol, and add to the SPE cartridge as eluent.

11.15.27.10. Repeat with another 5 mL of 0.3% NH_4OH/methanol.

11.15.27.11. Collect the 10 mL of eluent and concentrate per Section 11.6.

Note: *If the extracts will not be concentrated elute extract with a total of 8 mL (2 4 mL rinses) of 0.3% NH_4OH/methanol.*

11.16. Other Types of Sample Cleanup

11.16.1. Freezing technique to remove lipids.

If samples contain lipids then freeze the methanolic extract and QC extracts at -20^{o}C for at least 1 hour. Collect the solvent layer.

11.16.2. Cleanup with graphitized carbon will be applied to all samples as needed but is required for all DoD/DOE extracts.

11.16.2.1. Add 100 mg of graphitized carbon to each sample extract and QC extracts.

11.16.2.2. Shake vigorously and then let sit for 10 minutes.

11.16.2.3. Centrifuge each sample for 2 minutes at 1000 rpm.

11.16.2.4. Decant the solvent layer.

11.16.2.5. Proceed to Section 11.6, 11.7 or 11.12 as applicable.

11.17. AFFF Sample Preparation

11.17.1. QC for AFFF samples consists of a method blank, a laboratory control sample and a sample or matrix duplicate only. No matrix spike or matrix spike duplicate is needed.

11.17.2. Perform a 1,000,000 X serial dilution of the AFFF sample. Dilute 1 mL of AFFF sample to 1L with laboratory supplied water. Then dilute 1mL of this dilution to 1L with laboratory supplied water.

11.17.2.1. Be sure to retain all dilutions should the initial analysis warrant re-analysis at higher concentration.

11.17.3. Subsample 2.0 mL of this dilution and fortify with 0.5 mL IDA solution and 0.5mL of IS (50 ng/mL) solution: then add 7.0 mL of methanol.

11.17.4. Transfer a portion of the sample to a 300 uL polypropylene autosampler vial (7 drop-wise or approximately ½ filled is sufficient). Archive the rest of the sample for re-injection or dilution.

11.18. Instrument Analysis

Suggested operating conditions are listed in Tables 1-7 for the Waters and SCIEX LCMS systems:

Table 1 - Recommended Instrument Operating Conditions				
HPLC Conditions (Shimadzu HPLC)				
Column (Column temp = 45°C)	Phenomenex Gemini 3 µm C18 110Å, 50 X 2 mm			
Mobile Phase Composition	A = 20 mM Ammonium Acetate in Water B = Methanol			
Gradient Program	**Time**	**%A**	**%B**	**Flow Rate - mL/min**
	0	90	10	0.60
	0.1	45	55	0.60
	4.5	1	99	0.60
	4.95	1	99	0.60
	5	90	10	0.60
	Maximum pressure limit = 5,000 psi			
Injection Size	2 µL (fixed amount throughout the sequence). If non-concentrated extract then use 20 uL.			
Run Time	~6.6 minutes			
Mass Spectrometer Interface Settings (SCIEX 5500)				
MS Interface Mode	ESI Negative Ion. Minimum of 10 scans/peak.			
Ion Spray Voltage (kV)	4.5			
Entrance Potential (V)	5			
Declustering Potential (V)	25			
Desolvation Temp	600ºC			
Curtain Gas	35 psi			
Collision Gas	8 psi			

Table 2 - Recommended Instrument Operating Conditions								
Mass Spectrometer Scan Settings (SCIEX 5500)								
Compound	Comments	Reaction (MRM)	Dwell (sec)	Ent. Pot. (V)	Col. Energy (V)	Declu. Pot. (V)	Cell Exit Pot. (V)	Typ RT (Min)
PFBA	Native analyte	212.9 > 169	0.011	-5	-12	-25	-31	1.74
13C4-PFBA	IDA	217 > 172	0.011	-5	-12	-25	-31	1.74
PFBS	Native analyte	298.9 > 80	0.011	-6	-58	-55	-37	1.76
PFBS_2	Native analyte	298.9 > 99	0.011	-5	-40	-55	-12	1.76

Table 2 - Recommended Instrument Operating Conditions								
Mass Spectrometer Scan Settings (SCIEX 5500)								
Compound	Comments	Reaction (MRM)	Dwell (sec)	Ent. Pot. (V)	Col. Energy (V)	Declu. Pot. (V)	Cell Exit Pot. (V)	Typ RT (Min)
13C3-PFBS	IDA	301.9 > 83	0.011	-5	-40	-55	-12	1.76
PFPeA	Native analyte	262.9 > 219	0.011	-7	-12	-20	-34	1.99
13C5-PFPeA	IDA	267.9 > 223	0.011	-7	-12	-20	-35	1.99
4:2 FTS	Native analyte	327 > 307	0.011	-7	-32	-50	-10	2.06
M2-4:2FTS	IDA or Reverse Surrogate for TOP	329 > 81	0.011	-7	-32	-50	-10	2.06
PFHxA	Native analyte	313 > 269	0.011	-5	-12	-25	-37	2.25
PFHxA_2	Native analyte	313 > 119	0.011	-5	-12	-25	-37	2.25
13C2-PFHxA	IDA	315 > 270	0.011	-5	-12	-25	-38	2.25
PFHpA	Native analyte	363 > 319	0.011	-6	-12	-25	-41	2.57
PFHpA_2	Native analyte	363 > 169	0.011	-6	-12	-25	-41	2.57
13C4-PFHpA	IDA	367 > 322	0.011	-6	-12	-25	-41	2.57
PFPeS	Native analyte	349 > 80	0.011	-9	-66	-57	-40	2.15
PFPeS_2	Native analyte	349 > 99	0.011	-9	-40	-57	-12	2.15
PFHxS	Native analyte	399 > 80	0.011	-12	-74	-60	-43	2.59
PFHxS_2	Native analyte	399 > 99	0.011	-12	-74	-60	-43	2.59
18O2-PFHxS	IDA	403 > 84	0.011	-12	-74	-60	-43	2.59
6:2 FTS	Native analyte	427 > 407	0.011	-7	-32	-50	-10	2.91
M2-6:2FTS	IDA	429 > 81	0.011	-7	-32	-50	-10	2.91
PFOA	Native analyte	413 > 369	0.011	-6	-14	-25	-44	2.93
PFOA_2	Native analyte	413 > 169	0.011	-5	-22	-25	-12	2.93
13C4-PFOA	IDA	417 > 372	0.011	-6	-14	-25	-44	2.93
13C2-PFOA	IS	415 > 370	0.011	-6	-14	-25	-44	2.93
PFHpS	Native analyte	449 > 80	0.011	-11	-88	-65	-46	2.94
PFHpS_2	Native analyte	449 > 99	0.011	-11	-88	-65	-46	2.94
PFNA	Native analyte	463 > 419	0.011	-6	-14	-25	-47	3.29
PFNA_2	Native analyte	463 > 169	0.011	-6	-14	-25	-47	3.29
13C5-PFNA	IDA	468 > 423	0.011	-6	-14	-25	-48	3.29
PFOS	Native analyte	499 > 80	0.011	-9	-108	-65	-50	3.29
PFOS_2	Native analyte	499 > 99	0.011	-5	-58	-65	-12	3.29
PFNS	Native analyte	549 > 80	0.011	-10	-113	-75	-52	3.40
PFNS_2	Native analyte	549 > 99	0.011	-8	-71	-75	-12	3.40
PFDoS	Native analyte	699 > 80	0.011	-11	-76	-10	-11	4.48
PFDoS_2	Native analyte	699 >99	0.011	-11	-130	-10	-5	4.48
13C4-PFOS	IDA	503 > 80	0.011	-9	-108	-65	-50	3.29
PFDA	Native analyte	513 > 469	0.011	-6	-16	-25	-51	3.65
PFDA_2	Native analyte	513 > 169	0.011	-6	-16	-25	-51	3.65
13C2-PFDA	IDA	515 > 470	0.011	-6	-16	-25	-51	3.65
8:2 FTS	Native analyte	527 > 507	0.011	-7	-40	-50	-15	3.65
10:2 FTS	Native analyte	627 > 607	0.011	-7	-38	-110	-5	4.25

Table 2 - Recommended Instrument Operating Conditions								
Mass Spectrometer Scan Settings (SCIEX 5500)								
Compound	Comments	Reaction (MRM)	Dwell (sec)	Ent. Pot. (V)	Col. Energy (V)	Declu. Pot. (V)	Cell Exit Pot. (V)	Typ RT (Min)
M2-8:2FTS	IDA	529 > 81	0.011	-7	-40	-50	-15	3.65
PFOSA	Native analyte	498 > 78	0.011	-8	-85	-60	-50	3.7
13C8-PFOSA	IDA	506 > 78	0.011	-8	-85	-60	-50	3.7
N-MeFOSAA	Native analyte	570 > 419	0.011	-7	-36	-40	-15	3.82
d3-MeFOSAA	IDA	573 > 419	0.011	-7	-36	-40	-15	3.82
PFDS	Native analyte	599 > 80	0.011	-11	-118	-85	-54	3.96
PFDS_2	Native analyte	599 > 99	0.011	-11	-118	-85	-54	3.96
PFUdA	Native analyte	563 > 519	0.011	-7	-18	-25	-54	3.97
PFUdA_2	Native analyte	563 > 169	0.011	-7	-18	-25	-54	3.97
13C2-PFUdA	IDA	565 > 520	0.011	-7	-18	-25	-54	3.97
N-EtFOSAA	Native analyte	584 > 419	0.011	-7	-36	-50	-15	3.99
d5-EtFOSAA	IDA	589 > 419	0.011	-7	-36	-50	-15	3.99
PFDoA	Native analyte	613 > 569	0.011	-5	-18	-25	-54	4.3
PFDoA_2	Native analyte	613 > 169	0.011	-5	-18	-25	-54	4.3
13C2-PFDoA	IDA	615 > 570	0.011	-5	-18	-25	-54	4.3
PFTrDA	Native analyte	663 > 619	0.011	-7	-20	-25	-54	4.56
PFTrDA_2	Native analyte	663 > 169	0.011	-7	-20	-25	-54	4.56
PFTeDA	Native analyte	713 > 169	0.011	-2	-22	-25	-10	4.79
PFTeDA_2	Native analyte	713 > 219	0.011	-7	-36	-25	-30	4.79
13C2-PFTeDA	IDA	715 > 670	0.011	-2	-22	-25	-10	4.79
PFHxDA	Native analyte	813 > 769	0.011	-7	-24	-25	-54	5.25
PFHxDA_2	Native analyte	813 > 169	0.011	-7	-24	-25	-54	5.25
13C2-PFHxDA	IDA	815 > 770	0.011	-7	-24	-25	-54	5.25
PFODA	Native analyte	913 > 869	0.011	-7	-26	-25	-54	5.55
PFODA_2	Native analyte	913 > 169	0.011	-7	-26	-25	-54	5.55

Table 3 - Recommended Instrument Operating Conditions								
Mass Spectrometer Scan Settings (SCIEX 5500) for Fluorinated Replacement Chemicals								
Compound	Comments	Reaction (MRM)	Dwell (sec)	Ent. Pot. (V)	Col. Energy (V)	Declu. Pot. (V)	Cell Exit Pot. (V)	Typ RT (Min)
HFPO-DA	Native analyte	329.1 > 285	0.011	-10	-6	-48	-17	2.06
13C3-HFPO-DA	IDA	332.1 > 287	0.011	-10	-10	-40	-17	2.06
9Cl-PF3ONS (F53B major)	Native analyte	531 > 351	0.011	-10	-30	-120	-17	3.23

Table 3 - Recommended Instrument Operating Conditions Mass Spectrometer Scan Settings (SCIEX 5500) for Fluorinated Replacement Chemicals								
Compound	Comments	Reaction (MRM)	Dwell (sec)	Ent. Pot. (V)	Col. Energy (V)	Declu. Pot. (V)	Cell Exit Pot. (V)	Typ RT (Min)
11Cl-PF3OUdS (F53B minor)	Native analyte	631 > 451	0.011	-10	-40	-160	-17	3.84
Dona	Native analyte	377 > 251	0.011	-10	-16	-55	-17	2.33
Dona_2	Native analyte	377 > 85	0.011	-10	-35	-55	-17	2.33

Table 4 - Retention Times & Quantitation (SCIEX 5500)				
Native Compounds	**Typical Native RT (minutes)**	**IDA analog**	**Typical IDA RT (minutes)**	**Quantitation Method**
PFBA	1.54	13C4-PFBA	1.54	Isotope Dilution
PFPeA	1.56	13C5-PFPeA	1.56	Isotope Dilution
PFBS	1.78	13C3-PFBS	1.78	Isotope Dilution
PFHxA	2.03	13C2-PFHxA	2.03	Isotope Dilution
PFPeS	2.06	13C3-PFBS	1.78	Isotope Dilution
PFHpA	2.36	13C4-PFHpA	2.36	Isotope Dilution
PFHxS	2.37	18O2-PFHxS	2.37	Isotope Dilution
PFOA	2.71	13C4-PFOA	2.71	Isotope Dilution
PFHpS	2.72	13C4-PFOS	3.09	Isotope Dilution
PFNA	3.09	13C5-PFNA	3.09	Isotope Dilution
PFOS	3.09	13C4-PFOS	3.09	Isotope Dilution
PFNS	3.40	13C4-PFOS	3.09	Isotope Dilution
PFDA	3.45	13C2-PFDA	3.45	Isotope Dilution
FOSA	3.43	13C8-FOSA	3.43	Isotope Dilution
PFDS	3.77	13C4-PFOS	3.09	Isotope Dilution
PFUdA	3.78	13C2-PFUdA	3.78	Isotope Dilution
PFDoA	4.07	13C2-PFDoA	4.07	Isotope Dilution
PFTrDA	4.34	13C2-PFDoA	4.07	Isotope Dilution
PFDoS	4.48	13C4-PFOS	3.09	Isotope Dilution
PFTeDA	4.58	13C2-PFTeDA	4.58	Isotope Dilution
PFHxDA	4.99	13C2-PFHxDA	4.99	Isotope Dilution
PFODA	5.34	13C2-PFHxDA	4.99	Isotope Dilution
EtFOSAA	3.78	d5-EtFOSAA	3.78	Isotope Dilution
MeFOSAA	3.61	d3-MeFOSAA	3.60	Isotope Dilution
4:2 FTS	1.98	M2-4:2 FTS (If TOP then 13C-PFBS)	1.78	Isotope Dilution
6:2FTS	2.69	M2-6:2FTS	2.69	Isotope Dilution
8:2FTS	3.44	M2-8:2FTS	3.44	Isotope Dilution
HFPO-DA	2.06	13C3-HFPO-DA	2.06	Isotope Dilution

Table 4 - Retention Times & Quantitation (SCIEX 5500)				
Native Compounds	**Typical Native RT (minutes)**	**IDA analog**	**Typical IDA RT (minutes)**	**Quantitation Method**
9Cl-PF3ONS (F53B major)	3.23	13C4-PFOS	3.09	Isotope Dilution
11Cl-PF3OUdS (F53B minor)	3.84	13C4-PFOS	3.09	Isotope Dilution
Dona	2.33	13C4-PFOS	3.09	Isotope Dilution
10:2 FTS	4.25	M2-8:2 FTS	3.44	Isotope Dilution

Table 5 - Recommended Instrument Operating Conditions						
HPLC Conditions (Waters Acquity UPLC)						
Column (Column temp = 50°C)	Waters Acquity BEH 1.7µm C18, 3.0 x 150 mm					
Mobile Phase Composition	A = 20 mM Ammonium Acetate in Water B = Methanol					
Gradient Program	**Time**	**%A**	**%B**	**Curve**	**Flow Rate - mL/min.**	
	0	98	2	6	0.30	
	1	98	2	6	0.30	
	2	50	50	6	0.30	
	12	10	90	6	0.30	
	12.5	0	100	6	0.30	
	16	0	100	6	0.30	
	16.2	98	2	6	0.30	
	Maximum pressure limit = 15,000 psi					
Injection Size	10 µL (fixed amount throughout the sequence)					
Run Time	~20 minutes					
Mass Spectrometer Interface Settings (Quattro Premier XE)						
MS Interface Mode	ESI Negative Ion. Minimum of 10 scans/peak.					
Capillary (kV)	2.8					
Cone (V)	Varies from 8.0 to 65					
Extractor (V)	3					
Source Temp	135ºC					
Desolvation Temp	350ºC					
Cone Gas (nitrogen) Flow	25 L/hour					
Desolvation Gas (nitrogen) Flow	1100 L/hour					

Table 6 - Recommended Instrument Operating Conditions						
Mass Spectrometer Scan Settings (Quattro Premier XE)						
Compound	Comments	Reaction (MRM)	Dwell (sec)	Cone Volt.	Col. Energy	Function Number
PFBA	Native analyte	213 > 169	0.02	8	10	1
13C4-PFBA	IDA	217 > 172	0.02	12	10	1
PFPeA	Native analyte	263 > 219	0.02	10	10	2
13C5-PFPeA	IDA	268 > 223	0.02	11	9	2
PFBS	Native analyte	299 > 80	0.02	45	35	2
PFBS_2	Native analyte	299 > 99	0.02	45	35	2
13C3-PFBS	IDA	302 > 83	0.02	45	35	2
PFHxA	Native analyte	313 > 269	0.02	10	10	3
PFHxA_2	Native analyte	313 > 119	0.02	10	10	3
13C2-PFHxA	IDA	315 > 270	0.02	12	9	3
PFHpA	Native analyte	363 > 319	0.02	10	10	4
PFHpA_2	Native analyte	363 > 169	0.02	10	10	4
13C4-PFHpA	IDA	367 > 322	0.02	12	10	4
PFHxS	Native analyte	399 > 80	0.02	55	35	4
PFHxS_2	Native analyte	339 > 99	0.02	55	35	4
18O2-PFHxS	IDA	403 > 84	0.02	50	40	4
PFOA	Native analyte	413 > 369	0.02	12	10	5
PFOA_2	Native analyte	413 > 169	0.02	12	10	5
13C2-PFOA	IS	415 > 370	0.02	12	12	5
13C4-PFOA	IDA	417 > 372	0.02	12	12	5
PFHpS	Native analyte	449 > 80	0.02	60	38	5
PFHpS_2	Native analyte	449 > 99	0.02	60	38	5
PFNA	Native analyte	463 > 419	0.02	16	10	7
PFNA_2	Native analyte	463 > 169	0.02	16	10	7
13C5-PFNA	IDA	468 > 423	0.02	12	12	7
PFOS	Native analyte	499 > 80	0.02	60	40	6
PFOS_2	Native analyte	499 > 99	0.02	60	40	6
PFNS	Native analyte	549 > 80	0.02	60	40	6
PFNS_2	Native analyte	549 > 99	0.02	60	40	6
13C4-PFOS	IDA	503 > 80	0.02	35	48	6
PFDA	Native analyte	513 > 469	0.02	16	12	8
PFDA_2	Native analyte	513 > 169	0.02	16	12	8
13C2-PFDA	IDA	515 > 470	0.02	14	12	8
PFUdA	Native analyte	563 > 519	0.02	15	12	10
PFUdA_2	Native analyte	563 > 169	0.02	15	12	10

Table 6 - Recommended Instrument Operating Conditions						
Mass Spectrometer Scan Settings (Quattro Premier XE)						
Compound	Comments	Reaction (MRM)	Dwell (sec)	Cone Volt.	Col. Energy	Function Number
13C2-PFUdA	IDA	565 > 520	0.02	14	12	10
PFDS	Native analyte	599 > 80	0.02	74	48	10
PFDS_2	Native analyte	559 > 99	0.02	74	48	10
FOSA	Native analyte	498 > 78	0.02	40	32	9
13C8-FOSA	IDA	506 > 78	0.02	48	32	9
PFDoA	Native analyte	613 > 569	0.02	15	14	11
PFDoA_2	Native analyte	613 > 169	0.02	15	14	11
13C2-PFDoA	IDA	615 > 570	0.02	16	12	11
PFTrDA	Native analyte	663 > 619	0.02	12	12	11
PFTrDA_2	Native analyte	663 > 169	0.02	12	12	11
PFTeDA	Native analyte	713 > 169	0.02	12	18	11
PFTeDA_2	Native analyte	713 > 219	0.02	12	18	11
13C2-PFTeDA	IDA	715 > 670	0.02	15	15	11
PFHxDA	Native analyte	813 > 769	0.02	18	15	12
PFHxDA_2	Native analyte	813 > 169	0.02	18	15	12
PFODA	Native analyte	913 > 869	0.02	20	16	12
PFODA_2	Native analyte	913 > 169	0.02	20	16	12
13C2-PFHxDA	IDA	815 > 770	0.02	18	15	12
EtFOSAA	Native analyte	584 > 419	0.02	35	20	9
d5-EtFOSAA	IDA	589 > 419	0.02	30	25	9
MeFOSAA	Native analyte	570 > 419	0.02	30	28	9
d3-MeFOSAA	IDA	573 > 419	0.02	30	25	9
4:2FTS	Native analyte	327 > 307	0.02	40	30	5
M2-4:2FTS	IDA or Reverse Surrogate for TOP	329 > 81	0.02	40	30	5
6:2FTS	Native analyte	427 > 407	0.02	40	30	5
M2-6:2FTS	IDA	429 > 81	0.02	40	28	5
8:2FTS	Native analyte	527 > 507	0.02	40	28	8
M2-8:2FTS	IDA	529 > 81	0.02	40	28	8

Table 7 - Recommended Instrument Operating Conditions				
Retention Times & Quantitation (Quattro Premier XE)				
Native Compounds	**Typical Native RT (minutes)**	**IDA analog**	**Typical IDA RT (minutes)**	**Quantitation Method**
PFBA	4.77	13C4-PFBA	4.79	Isotope Dilution

Table 7 - Recommended Instrument Operating Conditions				
Retention Times & Quantitation (Quattro Premier XE)				
Native Compounds	**Typical Native RT (minutes)**	**IDA analog**	**Typical IDA RT (minutes)**	**Quantitation Method**
PFPeA	5.90	13C5-PFPeA	5.92	Isotope Dilution
PFBS	6.01	13C3-PFBS	6.01	Isotope Dilution
PFHxA	7.22	13C2-PFHxA	7.25	Isotope Dilution
PFPeS	7.20	18O2-PFHxS	8.64	Isotope Dilution
PFHpA	8.57	13C4-PFHpA	8.59	Isotope Dilution
PFHxS	8.60	18O2-PFHxS	8.64	Isotope Dilution
PFOA	9.80	13C4-PFOA	9.83	Isotope Dilution
PFHpS	9.80	13C4-PFOS	10.90	Isotope Dilution
PFNA	10.88	13C5-PFNA	10.92	Isotope Dilution
PFOS	10.87	13C4-PFOS	10.90	Isotope Dilution
PFNS	11.70	13C4-PFOS	10.90	Isotope Dilution
PFDA	11.82	13C2-PFDA	11.86	Isotope Dilution
FOSA	12.41	13C8-FOSA	12.46	Isotope Dilution
PFDS	12.57	13C4-PFOS	10.90	Isotope Dilution
PFUdA	12.62	13C2-PFUdA	12.66	Isotope Dilution
PFDoA	13.32	13C2-PFDoA	13.34	Isotope Dilution
PFTrDA	13.91	13C2-PFDoA	13.34	Isotope Dilution
PFTeDA	14.39	13C2-PFTeDA	14.39	Isotope Dilution
PFHxDA	15.16	13C2-PFHxDA	15.16	Isotope Dilution
PFODA	15.57	13C2-PFHxDA	15.16	Isotope Dilution
EtFOSAA	12.63	d5-EtFOSAA	12.62	Isotope Dilution
MeFOSAA	12.3	d3-MeFOSAA	12.28	Isotope Dilution
4:2FTS	7.02	M2-4:2 FTS (If TOP then 13C-PFBS)	6.01	Isotope Dilution
6:2FTS	10.08	M2-6:2FTS	10.08	Isotope Dilution
8:2FTS	11.95	M2-8:2FTS	11.95	Isotope Dilution

11.18.1. Post Spike Sample Analysis for AFFF samples

11.18.1.1. This section only applies to aqueous samples prepared by serial dilution instead of SPE that have reported value of <LOQ (RL) for any analyte.

11.18.1.2. Spike aliquots of the sample at the final dilution reported for the sample with all analytes that have reported of <LOQ in the final dilution. The spike must be at the LOQ concentration to be reported with the sample (the < LOQ value).

11.18.1.3. When analyte concentrations are calculated as <LOQ, the spike must recover within 70-130% of its true value.

11.18.1.4. It the recovery does not meet this criteria, the sample, sample duplicate and post spike sample must be reanalyzed at consecutively higher dilutions until the criteria is met.

11.18.2. Tune and calibrate the instrument as described in Section 10.

11.18.3. A typical run sequence is as follows:

- Rinse Blank (RB, not linked to anything)
- Start ICAL with CCVL but called IC in TALS (starts the 12 hour clock or time 0:00)
- Rest of ICAL
- ICB: link to midpoint of ICAL and samples
- ICV: link to midpoint of ICAL and samples (If ICAL good)
- CCB: link to midpoint of ICAL and samples
- PFOA RT marker
- Rinse Blank (RB, not linked to anything)
- 10 samples: link to midpoint of ICAL
- CCV: link to midpoint of ICAL
- 10 more samples: link to midpoint of ICAL
- CCV: link to midpoint of ICAL
- Etc.
- CCVL (within 12 hours from CCVL in ICAL, can be the ending CCV and starts 12 hours all over again): if this occurs link to the midpoint of the ICAL/toggle it as opening/closing CCV.
- CCV: link to midpoint of ICAL
- 10 samples: link to midpoint of ICAL
- CCV: link to midpoint of ICAL
- If no ICAL run that day
- CCB: link to CCVIS
- CCVL (starts 12 hour clock): link to CCVIS
- CCVIS: link to midpoint of ICAL
- 10 samples: link to CCVIS
- CCV: link to CCVIS
- 10 samples: link to CCVIS
- CCV: link to CCVIS
- Etc.

- If going over 12 hours in the sequence: CCVL (within 12 hours from CCVL at item 2 above, can be the ending CCV and starts 12 hours all over again): if this occurs link to the CCVIS /toggle as opening and closing CCV.
- CCV: link to CCVIS
- 10 samples: link to CCVIS
- CCV: link to CCVIS

12. CALCULATIONS

12.1. If the concentration of the analyte ions exceeds the working range as defined by the calibration standards, then the sample must be diluted and reanalyzed. It may be necessary to dilute samples due to matrix.

12.2. Qualitative Identification

12.2.1. The retention times of PFAS with labeled standards should be the same as that of the labeled IDA's to within 0.05 min. For PFAS with no labeled standards, the RT must be within ± 0.3 minutes of the ICV and CCV standards. ***Note**: The IDA RT and native RT may be offset by 0.02 to 0.04 minutes.*

12.3. The ICAL established in Section 10 is used to calculate concentrations for the extracts.

12.4. Extract concentrations are calculated as below. The first equation applies to the linear fit, the second to the quadratic line fit.

Equation 3

$$\text{Concentration, ng/mL} = \frac{y - c}{b}$$

Equation 4

$$\text{Concentration, ng/mL} = \frac{-b + \sqrt{b^2 - 4a(c - y)}}{2a}$$

Where:

y = $\frac{\text{Area (analyte)}}{\text{Area (IS)}} \times \text{Concentration (IS)}$

x = concentration
a = curvature
b = slope
c = intercept

12.5. Water Sample Result Calculation:

Equation 5

$$\text{Concentration, ng/L} = \frac{C_{ex} V_t}{V_o}$$

Where:

C_{ex}	=	Concentration measured in sample extract (ng/mL)
V_t	=	Volume of total extract (mL)
V_o	=	Volume of water extracted (L)

12.6. Soil Sample Result Calculation:

Equation 6

$$\text{Concentration, } ng/g = \frac{C_{ex} V_t}{W_s D}$$

Where ng/g = µg/kg and:

C_{ex}	=	Concentration measured in sample extract (ng/mL)
V_t	=	Volume of total extract (mL)
W_s	=	Weight of sample extracted (g)
D	=	Fraction of dry solids, which is calculated as follows:

$$\frac{100 - \%\,\text{moisture in sample}}{100} \quad \text{(for dry weight result)}$$

12.7. IDA Recovery Calculation:

Equation 7

$$\%\,\text{Recovery} = \frac{A_t Q_{is}}{A_{is} Q_t RRF_{IDA}} X 100$$

Where ng/g = µg/kg and:

RF_{IDA}	=	Response Factor for IDA compound
A_t	=	Area response for IDA compound
A_{IS}	=	Area Response for IS compound
Q_{IS}	=	Amount of IS added
Q_t	=	Amount of IDA added

12.8. Raw data, calibration summaries, QC data, and sample results are reviewed by the analyst. These must also be reviewed thoroughly by a second qualified person. See the Data Review Policy (WS-PQA-0012). These reviews are documented on the Data Review Checklist.

13. METHOD PERFORMANCE

13.1. The group/team leader has the responsibility to ensure that this procedure is performed by an associate who has been properly trained in its use and has the required expertise.

13.2. Method Detection Limit

The laboratory must generate a valid method detection limit for each analyte of interest. The MDL must be below the reporting limit for each analyte. The procedure for determination of the method detection limit is given in 40 CFR Part 136, Appendix B, and further defined in SOP WS-QA-0006 and policy WS-PQA-003. MDLs are available in the Quality Assurance Department.

13.3. Initial Demonstration of Capability (IDOC)

Each analyst performing this procedure must successfully analyze four LCS QC samples using current laboratory LCS control limits. IDOCs are approved by the Quality Assurance Manager and the Technical Director. IDOC records are maintained by the QA staff in the central training files.

13.4. The laboratory must generate a valid method detection limit for each analyte of interest. The MDL must be below the reporting limit for each analyte. The procedure for determination of the method detection limit is given in 40 CFR Part 136, Appendix B, and further defined in WS-QA-0006 and policy WS-PQA-003.

14. POLLUTION PREVENTION

14.1. All waste will be disposed of in accordance with Federal, State and Local regulations.

14.2. Solid phase extraction used for water samples greatly reduces the amount of solvent used compared to liquid-liquid extraction.

14.3. Standards and reagents are purchased and prepared in volumes consistent with laboratory use to minimize the volume of expired standards and reagents requiring disposal.

14.4. Where reasonably feasible, technological changes have been implemented to minimize the potential for pollution of the environment. Employees will abide by this method and the policies in Section 13 of the Corporate Safety Manual for “Waste Management and Pollution Prevention.”

14.5. Do not allow waste solvent to vent into the hoods. All solvent waste is stored in capped containers unless waste is being transferred.

14.6. Transfer waste solvent from collection cups (tri-pour and similar containers) to jugs and/or carboys as quickly as possible to minimize evaporation.

15. WASTE MANAGEMENT

The following waste streams are produced when this method is carried out:

15.1. Assorted test tubes, autovials, syringes, filter discs and cartridges. Dump the solid waste into a yellow contaminated lab trash bucket. When the bucket is full or after no more than one year, tie the plastic bag liner shut and put the lab trash into the hazardous waste – landfill steel collection drum in the H3 closet. When the drum is full or after no more than 75 days, move it to the waste collection area for shipment.

15.2. Extracted soil samples, used sodium sulfate, paper funnel filters, glass wool, thimbles, and extracted solids saturated with solvents. Dump these materials into an orange contaminated lab trash bucket. When the bucket is full or after no more than one year, tie the plastic bag liner shut and put the lab trash into the incineration steel collection drum in the H3 closet. When the drum is full or after no more than 75 days, move it to the waste collection area for shipment.

15.3. Waste Methanol. Collect the waste solvents in tripours during use. Empty the tripours into a 1-liter to 4-liter carboy at the fume hood. When the carboy is full, or at the end of your shift, whichever comes first, empty the carboy into the steel flammable solvent drum in the H3 closet. When full to no less than six inches of the top, or after no more than 75 days, move the steel flammable solvent drum to the waste collection area for shipment.

15.4. Mixed water/methanol waste from soil extraction. Collect the waste in the HPLC waste carboy. When full, or after no more than one year, dump into the blue plastic HPLC collection drum in the H3 closet. When the drum is full, to no less than six inches of the top, or after no more than 75 days, move it to the waste collection area for shipment.

15.5. Aqueous acidic waste from the LCMS instrument contaminated with methanol. This is collected in a 1-gallon carboy at the instrument. When the carboy is full, or after no more than one year, it is emptied into the blue plastic HPLC collection drum in the H3 closet. When the drum is full to between two and six inches of the top, or after no more than 75 days, move it to the waste collection area for shipment.

15.6. Autovials contaminated with methanol. As the autovials are removed from the instrument after analysis, they are collected in open containers at the instrument. After all autovials are removed, the open container must be dumped into a closed satellite collection container in a fume hood, as the punctured septa in the autovial can allow methanol and other contaminants to evaporate into the atmosphere. The satellite collection containers are transferred to the waste disposal area when full or after no more than one year, where they are disposed through the vial eater.

16. REFERENCES

16.1. Cheryl Moody, Wai Chi Kwan, Johnathan W. Martin, Derek C. G. Muir, Scott A. Mabury, “Determination of Perfluorinated Surfactants in Surface Water Samples by

Two Independent Analytical Techniques: Liquid Chromatography/Tandem Mass Spectrometry and 19FNMR," Analytical Chemistry 2001, 73, 2200-2206.

16.2. John Giesy et al., "Accumulation of Perfluorooctane Sulfonate in Marine Mammals", Environmental Science & Technology, 2001 Vol. 35, No. 8, pages 1593-1598.

16.3. U.S. EPA, "Residue Chemistry Test Guidelines, OPPTS 860.1340, Residue Analytical Method", EPA 712-C-95-174, August 1995.

16.4. STL Denver White Paper DEN-W-LC-002, "Method Validation Study for Analysis of Ammonium Perfluorooctanate in Soil Matrices by High Performance Liquid Chromatography/Mass Spectrometry (HPLC/MS/MS)", Mark Dymerski, September 5, 2003.

16.5. STL Denver White Paper DEN-W-LC-003, "Addendum A to Method Validation Study for Analysis of Ammonium Perfluorooctanate in Soil Matrices by High Performance Liquid Chromatography/Mass Spectrometry (HPLC/MS/MS)", Mark Dymerski, August 6, 2003.

16.6. STL Denver White Paper DEN-W-LC-004, "Method Validation Study for Analysis of Perfluorooctanoic Acid in Waters by High Performance Liquid Chromatography/Tandem Mass Spectrometry (HPLC/MS/MS)", Mark Dymerski, January 26, 2005.

16.7. Waters application note; "Acquity UPLC System for Quantifying Trace Levels of Perfluorinated Compounds with an Acquity PFC Analysis Kit", Peter J. Lee, Evan T. Bernier, Gordon T. Fujimoto, Jeremy Shia, Michael S. Young, and Alice J. Di Gloia, Waters Corporation, Milford, MA. USA.

16.8. US EPA, "Method 537 - Determination of Selected Perfluorinated alkyl acids in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometery (LC/MS/MS)", Version 1.1, September 2009, J.A. Shoemaker, P.E. Grimmett, B.K. Boutin, EPA Document #: EPA/600/R-08/092

16.9. Erika F. Houtz and David L. Sedlak, "Oxidative Conversion as a Means of Detecting Precursors to Perfluoroalkyl Acids in Urban Runoff," Environmental Science and Technology 46, no. 17 (2012): 9342-49.

17. METHOD MODIFICATIONS

17.1. Modifications from Method 537 are detailed below:

17.1.1. Water sample containers are not preserved with Trizma.

17.1.2. The method has been modified to address soil/solid matrices. The extraction

holding time is set at 14 days.

17.1.3. The analyte list has been expanded. The number of labeled analytes has been expanded as well to improve quantitation.

17.1.4. The reporting limits differ as they are all set at one consistent value.

17.1.5. Calibration levels differ from the referenced method.

17.1.6. More labeled analytes are fortified into the samples prior to the extraction process. Most target analytes are quantitated against a labeled analyte.

17.1.7. There is no symmetry requirement.

17.1.8. Calibration, both initial and continuing, has different acceptance criteria due to the longer list of analytes, and the use of isotope dilution quantitation.

17.1.9. The eluents and HPLC configuration differs. As a result the final extract is in 80:20 methanol:water.

17.1.10. The LCS and MS/MSD are spiked at one concentration and do not rotate between a low to high levels.

17.1.11. Samples are not checked for residual chlorine or pH.

17.1.12. A different SPE cartridge (Waters OASIS WAX) is used for the extraction process. As a result solvents and elution procedures are different.

18. ATTACHMENTS

18.1. Attachment 1 - Analysis of Perfluorinated Compounds (PFAS) in Water via In Line Solid Phase Extraction (SPE).

19. REVISION HISTORY

Revisions to Attachment 1 are documented in the attachment.

Revisions prior to 05/01/2017 have been removed and are available in previous versions of this SOP.

19.1. WS-LC-0025, Revision 3.5, Effective 02/27/2019

19.1.1. Added Section 11.3.6, “After the entire sample has been loaded onto the column, rinse the sample bottle with two 5 mL aliquots of reagent water and pour onto the column reservoir.”

19.1.2. Editorial changes.

19.2. WS-LC-0025, Revision 3.4, Effective 02/13/2019

19.2.1. Section 6.4 added, "The average weight of the HDPE bottles with HDPE screw caps are calibrated once a year. The calibration is performed by weighing 10 bottles with caps and dividing by 10 to get the average weight. The average weight is used in section (11.3.5.1.d)."

19.2.2. Section 7.4.1 revised, "an" to "every" and removed "or when a new column is installed".

19.2.3. Add Section 7.4.1.1, "Attach this document to the ICV from the associated ICAL by scanning the document and associating it to the file as a document type of High Res MS Tune in TALS. Use the following naming convention: "_ZbatchnumberTPFOA"."

19.2.4. Added Section 8.2.1, "Projects performed for the state of New Jersey have an analytical holding time 28 days from the extraction date."

19.2.5. Added Section 8.2.2, "For projects performed for the state of New Jersey a field reagent blank (FRB) must be collected with each sample set. Acceptance limits are <RL for each analyte."

19.2.6. Added Section 9.4.1, "Projects performed for the state of New Jersey: LCS (mid and high spike) recovery limits are 70-130%. Low level LCS recovery limits are 50-150%. The spike level must rotate between low, medium and high."

19.2.7. Added Section 9.5.1, "Projects performed for the state of New Jersey: MS/MSD (mid and high spike) recovery limits are 70-130%. Low level MS/MSD recovery limits are 50-150%. The spike level must rotate between low, medium and high."

19.2.8. Added Section 9.10, "TOP Oxidation Efficiency" and its associated subsections.

19.2.9. Added Section 9.11, "Ion Ratio" and associated subsections.

19.2.10. Added Section 10.8.2.6, "Projects performed for the state of New Jersey: MS/MSD (mid and high spike) recovery limits are 70-130%. Low level MS/MSD recovery limits are 50-150%. The spike level must rotate between low, medium and high."

19.2.11. Section 10.11.3 added, "and the state of New Jersey".

19.2.12. Added Section 10.12.5, "Projects performed for the state of New Jersey: All

analyte concentrations in the CCV must be within + 30% of their true value. All analyte concentrations in the low level CCV must be within + 50% of their true value."

19.2.13. Added Section 11.3.5.1, "If the SPE column should plug (flow rate <1 drop per minute) prior to the entire content of the sample container passing through the column do the following:" and its associated subsections.

19.2.14. Sections 11.14.15.8 and 11.15.27.8 removed, "with a flow rate of 1 mL/minute".

19.2.15. Section 11.18.3 removed, "(as needed)" from the PFOA RT marker.

19.2.16. Throughout SOP revised, "1 mL/minute" to "3-5 drops per second".

19.2.17. Editorial changes.

19.3. WS-LC-0025, Revision 3.3, Effective 12/03/2018

19.3.1. Added Section 6.9, "Phenomenex Gemini 3 µm C18 110A, 50 X 3 mm, Part No. 00B-4439-Y0."

19.3.2. Tables 2 and 6 revised comment for M2-4:2 FTS to, "IDA or Reverse Surrogate for TOP".

19.3.3. Tables 4 and 7 revised header from "IS Analog" to "IDA Analog", and revised "4:2 FTS" entry to "M2-4:2 FTS (If TOP then 13C-PFBS)".

19.3.4. Editorial changes.

19.4. WS-LC-0025, Revision 3.2, Effective 08/20/2018

19.4.1. Section 1 added, "1H,1H,2H,2H-perfluorododecane sulfonate" and "Perfluoro-1-dodecansulfonic acid" entries to table.

19.4.2. Section 1.2 revised table entry for "Adona" to "Dona".

19.4.3. Section 7.4 added, "PFDoS" and "10:2 FTS" entries to table.

19.4.4. Section 7.4 revised, "Adona" entry to "Dona".

19.4.5. Table 2 added, "PFDoS", "PFDoS_2", and "10:2 FTS" entries to table.

19.4.6. Table 3 revised, "Adona" and "Adona_2" entries to "Dona" and "Dona_2".

19.4.7. Table 4 added, "PFDoS" and "10:2 FTS" entries to table.

19.4.8. Table 4 revised, “Adona entry to “Dona”.

19.4.9. Editorial changes.

19.5. WS-LC-0025, Revision 3.1, Effective 06/21/2018

19.5.1. Section 11.2.1 revised to, “Visually inspect samples for the presence of settled and/or suspended sediment/particulates. If present or if the sample is biphasic add IDA prior to any sample decanting or centrifugation. If the sample requires decanting or centrifugation contact the client for guidance prior to such action. Decanting or filtering of the sample can lead to a low bias.”

19.5.2. Editorial changes.

19.6. WS-LC-0025, Revision 3.0, Effective 04/13/2018

19.6.1. Section 1.1 updated table with PFPeS and PFNS analytes.

19.6.2. Added Section 2.2, which details the analytes that can be covered by the method under special request.

19.6.3. Added Section 3.13, “AFFF: Aqueous Film Forming Foam”.

19.6.4. Section 6.19 added, “Create all eluents in Reagent module, label eluent containers with TALS label and place 2nd label into maintenance log when put into use” to table.

19.6.5. Section 7.1.2 added, “Prepared by weighing 1.509g of ammonium acetate and dissolving in 1L of water. The resultant solution is filtered through a 0.22um filter before use. This solution has volatile components, thus it should be replaced every 7 days or sooner.”

19.6.6. Section 7.1.3 added, “Prepared by diluting 12mL of ammonium hydroxide into 4L of methanol.”

19.6.7. Section 7.1.8 added, “Prepared by weighing 16g of potassium hydroxide and dissolving in 4L of methanol.”

19.6.8. Section 7.1.11 added, “Prepared by diluting 400mL of 1N NaOH into 3.6L of water for a total volume of 4L.”

19.6.9. Section 7.4 updated table with PFPeS and PFNS analytes.

19.6.10. Section 7.4, added table to detail ICAL for Fluorinated Replacement Compounds.

19.6.11. Added Section 8.1.1, "Water samples collected from a known chlorinated source should be preserved with Trizma."

19.6.12. Added Section 9.9.3, "If the IS does not meet criteria, re-analyze the extract. If the IS meets criteria in the second analysis, report that analysis. If the IS does not meet criteria in the second analysis, report the first analysis with narration."

19.6.13. Added Section 11.14.6, "Add 2g of potassium persulfate and 1.9 mL of 10N NaOH to each "Post" sample container."

19.6.14. Removed Section 11.14.8, "Add 2g of potassium persulfate and 1.9 mL of 10N NaOH to each "Post" sample container."

19.6.15. Added Section 11.14.9, "Cap each "Post" sample container, invert 2-3 times prior to placing container into water bath."

19.6.16. Added Section 11.5 and associated subsections, which detail the "TOPS (Total Oxidizable Precursor) Assay for Soil Sample".

19.6.17. Section 11.8 updated Table labeling, added PFPeS and PFNS analytes throughout Tables where applicable, and updated Table 7 to reflect current retention times and quantitation.

19.6.18. Section 11.8 added Table 6, "Recommended Instrument Operating Conditions Mass Spectrometer Scan Settings (SCIEX 5500) for Fluorinated Replacement Chemicals"

19.6.19. Section 11.18.3 removed outdated run sequence and replaced with current run sequence.

19.6.20. Editorial changes.

19.7. WS-LC-0025, Revision 2.9, Effective 11/22/2017

19.7.1. Section 1.2, table updated to reflect ranges after removing MeFOSA and EtFOSA from the SOP in the previous revision.

19.7.2. Section 9.3.6, last sentence changed to read, "Reprepare and reanalyze all field and QC samples associated with the contaminated method blank."

19.7.3. Section 9.7, first sentence changed to read, "Initial calibration verification (ICV) – A second source standard is analyzed with the initial calibration curve.

19.7.4. Section 1.3.1 revised to read, "Once the optimal mass assignments (within ±0.5 amu of true) are made immediately following the initial tune, the lowest level standard from the initial calibration curve is assessed to ensure that a signal to noise ratio greater than 10 to 1 (S/N > 10:1) is achieved for each PFAS analyte. The first level standard from the initial calibration curve is used to evaluate the tune stability on an ongoing basis. The instrument mass windows are set initially at ± 0.5 amu of the true value; therefore, continued detection of the analyte transition with S/N > 10:1 serves as verification that the assigned mass remains within ± 0.5 amu of the true value, which meets the DoD/DOE QSM tune criterion. For QSM work, the instrument sensitivity check (section 10.12.4) is also evaluated to ensure that the signal to noise criteria is met."

19.7.5. Editorial changes.

19.8. WS-LC-0025, Revision 2.8, Effective 11/06/2017

19.8.1. Revised Section 4.5 to "Both branched and linear PFAS isomers can potentially be found in the environment. Linear and branched isomers are known to exist for PFOS, PFOA, PFHxS, PFBS, EtFOSAA, and MeFOSAA based upon the literature. If multiple isomers are present for one of these PFAS they might be adjacent peaks that completely resolved or not, but usually with a deflection point resolved during peak integration. The later of these peaks match the retention time of its labeled linear analog. In general, earlier peaks are the branched isomers and are not the result of peak splitting.

At this time only PFOS, PFOA and PFHxS are commercially available as technical mixtures. These reference standards of the technical mixtures for these specific PFAS are used to ensure that all appropriate peaks are included during peak integration."

19.8.2. Sections 4.8 and 7.2.1.1, corrected the in-sample contributions to 0.30 ng/L and 0.015 ug/kg.

19.8.3. Removed Section 7.1.14, "Methanol-Water, 78:22 vol./vol., prepared by mixing 780 mL methanol and 220 mL reagent water. Stored in polypropylene bottle and sealed with polypropylene screw cap." Reagent was added incorrectly.

19.8.4. Section 7.2.4, corrected the factor to 0.956 from 1.046.

19.8.5. Added Section 7.4.1, "A technical (qualitative) grade PFOA standard which contains both linear and branched isomers is used as a retention time (RT) marker. This is used to integrate the total response for both linear and

branched isomers of PFOA in environmental samples while relying on the initial calibration with the linear isomer quantitative standard This technical (qualitative) grade PFOA standard is analyzed initially, after an initial calibration when a new column is installed or when significant changes are made to the HPLC parameters."

19.8.6. Section 9.7, added "Rerun the initial calibration" as the last bullet item.

19.8.7. Added Section 10.3.1, "The first level standard from the initial calibration curve is used to evaluate the tune criteria. The instrument mass windows are set at ±0.5 amu; therefore, detection of the analyte serves as verification that the assigned mass is within ±0.5 amu of the true value, which meets the DoD/DOE QSM tune criterion.

19.8.8. Section 10.10.1, appended "containing both IDA and IS" to the end of the paragraph.

19.8.9. Sections 11.6.3 and 11.12.2.3, changed "78:22 methanol:water" to "methanol".

19.8.10. Sections 1.1 and 7.4, removed EtFOSA and MeFOSA from tables due to low volume of requests for those analytes.

19.8.11. Removed Section 2.2.1, "Optional cleanups may include sample freezing and/or cleanup by SPE cartridge, unless EtFOSA and MeFOSA are requested."

19.8.12. Removed EtFOSA/MeFOSA specific comments in various sections throughout the document.

19.8.13. Section 7.4 Note added, "The concentration of the calibration solutions for non-concentrated extracts is 1/20th the levels indicated above."

19.8.14. Section 7.9, changed 1000 ng/mL to 250 ng/mL and replaced final sentence with "The internal standard solution used for the non-concentrated extracts is at a concentration of 50 ng/mL."

19.8.15. Removed Section 11.2.8, "If EtFOSA and/or MeFOSA are requested, add 100uL of IS and then adjust the final volume (FV) of these aliquots to 5.0 mL with MeOH. QC samples, LCS, MS, and MSD will require concentration via nitrogen to adjust the FV to 5.0 mL. Vortex each sample. Then, transfer a portion of the extract to a 300 uL polypropylene autosampler vial (7 drop-wise or approximately ½ filled is sufficient). Archive the rest of the extracts for re-injection and dilution."

19.8.16. Added Section 11.5.4, “Proceed to Section 11.15.2 (Graphitized Carbon Cleanup) as needed. This is required for all DoD/DOE extracts.”

19.8.17. Added Section 11.7.1.1, “Seal the test tube tightly. Invert container several times and then vortex. Allow extract to settle for 10 minutes prior to moving to the next step.”

19.8.18. Inserted Section 11.8.1.1, “Projects performed under the auspices of the DoD/DOE must have the entire sample homogenized prior to subsampling in accordance with QSM 5.1 criteria.”

19.8.19. Section 11.11.4, added “(Graphitized Carbon Cleanup) as needed. This is required for all DoD/DOE extracts.”

19.8.20. Section 11.14.6, added “Spike all “Pre” and “Post” samples with 25uL of the reverse surrogate solution (Section 7.8).”

19.8.21. Section 11.15.2, revised to read, “Cleanup with graphitized carbon will be applied to all samples as needed but is required for all DoD/DOE extracts.”

19.8.22. Added Section 11.15.2.5, “Proceed to Section 11.6, 11.7, or 11.12 as applicable.”

19.8.23. Removed Sections 11.15.3 through 11.15.6.

19.8.24. Added Section 11.16, “AFFF Sample Preparation”.

19.8.25. Section 11.17, removed EtFOSA, MeFOSA, d5-EtFOSA, and d3MeFOSA from all tables.

19.8.26. Section 11.17, changed masses for M2-4:2FTS, M2-6:2FTS, and M2-8:2FTS. Initially assigned daughter masses were bleeding through from the native analog.

19.8.27. Section 11.17, all tables on MS Interface Mode Line, added “Minimum of 10 scans/peak.”

19.8.28. Added Section 11.17.1, “Post Spike Sample Analysis for AFFF Samples”.

19.8.29. Added Section 11.8.4.1 “Spike non-concentrated samples at 0.5 mL of LCS/Matrix Spike Solution.”

19.8.30. Added Section 11.8.5.1, “Spike non-concentrated samples at 0.5 mL of IDA PFC Solution.”

19.8.31. Editorial changes.

19.9. WS-LC-0025, Revision 2.7, Effective 09/20/2017

19.9.1. Section 1.1 table, added 1H,1H,2H,2H-perfluorohexane sulfonate *(4:2)*.

19.9.2. Section 1.1, removed "Sample results for PFOA may also be reported as APFO, at the request of the client. (See Section 12.7)."

19.9.3. Section 1.2 and 11.8.2, updated tissue extracted mass and RL.

19.9.4. Section 2.5, removed "and assumes a proportional relationship between the initial calibration and the analyte in the extract. The ratio of the peak response to mass or concentration injected is used to prepare a calibration curve."

19.9.5. Added Section 6.6, "Extract concentrator or nitrogen manifold with water bath heating to 50-55°C".

19.9.6. Added Section 7.1.14, "Methanol-Water, 78:22 vol./vol., prepared by mixing 780 mL methanol and 220 mL reagent water. Stored in polypropylene bottle and sealed with polypropylene screw cap."

19.9.7. Section 7.2.1.1, revised "roughly 0.15 pg/L" to "roughly 0.15 ng/L".

19.9.8. Section 7.4 table, added:

4:2 FTS	0.5	1.0	2.0	20	50	200	400

19.9.9. Section 7.4 table, revised Labeled Isotope Dilution Analytes (IDA) Section.

19.9.10. Section 7.4 table, added:

Internal Standard (IS)							
13C2-PFOA	50	50	50	50	50	50	50

19.9.11. Section 7.4, removed "FOSAA may be added to the mix and are added at the same concentration as FOSA."

19.9.12. Added Section 7.9, "Internal Standard Solution, 1000 ng/mL. The internal standard solution is prepared by diluting 13C2-PFOA to produce a solution containing this compound at a concentration of 1000 ng/mL in methanol. This is added to all extracts prior to analysis. Non-concentrated extracts are fortified with a 5X dilution of this solution."

19.9.13. Section 8.1, changed "250 mL" to "8 oz."

19.9.14. Added Sections 9.3.6, 9.8.2.3, 10.10.4, 10.8.2.5, 10.11.3, and 10.12.4 to address DOD QSM 5.1 Table B-15 criteria.

19.9.15. Added Section 9.9, “Internal Standard.”

19.9.16. Updated all tables to indicate target analyte quantitation via isotope dilution. Internal standard quantitation is only used to quantitate the IDA recoveries.

19.9.17. Added Section 10.8.2.4, 10.12.2, and 10.12.2.1 to incorporate IS criteria into calibrations.

19.9.18. Section 11.2.1, “Evaluate if the sample can be decanted or centrifuged; if not, contact the client for guidance. Filtering the sample can lead to a low bias.”

19.9.19. Added Section 11.2.3.1, “Alternatively, weigh the sample container prior to extraction and then weigh the sample container after extraction to determine the initial volume.”

19.9.20. Added Section 11.5.3, “Note: If the extracts will not be concentrated elute extract with a total of 8 mL of 0.3% NH_4OH/methanol.”

19.9.21. Added Section 11.6.2.3, “Add 300 uL of the 78:22 methanol:water solution and mix the contents well using a vortex mixer.”

19.9.22. Added Section 11.6.2.4, “Add 100 uL of Internal Standard (IS) solution to each extract and vortex to mix.”

19.9.23. Added Section 11.7, “Final volume for non-concentrated extract”.

19.9.24. Revised Section 11.11, “SPE Elution of Solid Extracts”.

19.9.25. Revised Section 11.12, “Extract Concentration for Solid Samples”.

19.9.26. Removed Section 12.8, “If results are to be reported as ammonium perfluorooctanoate (APFO), instead of PFOA, apply a multiplier of 1.0406 to the sample results to correct for the molecular weight differences between PFOA and APFO or this adjustment can be made during the preparation of the standards used for calibration. (Use one, not both.)”

19.9.27. Removed Section 13.4 – it was a copy of Section 13.2.

19.9.28. Various revisions to fulfill requirements based on DOD/DOE QSM 5.1.

19.9.29. Editorial changes.

19.10. WS-LC-0025, Revision 2.6, Effective 08/15/2017

19.10.1. Section 7.4, added MPFBS, MPFTeDA, and MPFHxDA to the table.

19.10.2. Section 11.15, added 13C-PFBS to the Recommended Instrument Operating Conditions table for SCIEX 5500.

19.10.3. Section 11.15 Recommended Instrument Operating Conditions table, changed the mass transitions for native PFTeDA from 713 > 669 (quant) and 713 > 169 (qualifier) to 713 > 169 (quant) and 713 > 219 (qualifier).

19.10.4. Editorial changes.

19.11. WS-LC-0025, Revision 2.5, Effective 07/10/2017

19.11.1. Revised Section 11.6.1 to read "Prior to concentrating each sample, add 100 uL of water."

19.11.2. Revised Section 11.6.2 to read "Concentrate each sample under a gentle stream of nitrogen until the methanol is evaporated and the 100 uL of water remains.

11.6.2.1 This blow down must take a minimum of 3.5 hours.

11.6.2.2 Extracts can not remain in the water bath longer than 5 minutes once concentrated."

19.11.3. Revised Section 11.6.3 to read "Add 400 uL of methanol to each extract, soak, and vortex to mix well. This will create an extract with a final solvent composition of 80:20 methanol:water."

19.11.4. Revised Section 11.11.1 to read "Prior to concentrating each sample, add 200 uL of water."

19.11.5. Revised Section 11.11.2 to read "Concentrate each sample under a gentle stream of nitrogen until the methanol is evaporated and the 200 uL of water remains."

11.11.2.1 This blow down must take a minimum of 3.5 hours.

11.11.2.2 Extracts can not remain in the water bath longer than 5 minutes once concentrated."

19.11.6. Revised Section 11.11.3 to read "Add 800 uL of methanol to each extract, soak, and vortex to mix well. This will create an extract with a final solvent composition of 80:20 methanol:water."

1. SCOPE AND APPLICATION

1.1. This procedure describes the analysis of water samples via in line solid phase extraction (SPE) for the following compounds using liquid chromatography / tandem mass spectrometry (LC/MS/MS) on a SCIEX 5500.

Compound Name	Abbreviation	CAS #
Perfluoroalkylcarboxylic acids (PFCAs)		
Perfluoro-n-heptanoic acid	PFHpA	375-85-9
Perfluoro-n-octanoic acid	PFOA	335-67-1
Perfluoro-n-nonanoic acid	PFNA	375-95-1
Perfluorinated sulfonic acids (PFSAs)		
Perfluoro-1-butanesulfonic acid	PFBS	375-73-5
Perfluoro-1-hexanesulfonic acid	PFHxS	355-46-4
Perfluoro-1-octanesulfonic acid	PFOS	1763-23-1

1.2. The working range of the method is listed below. The linear range can be extended by diluting the extracts.

Matrix	Nominal Sample Size	Reporting Limit	Working Range
Water	1.0 mL	2.0 ng/L	2 to 200 ng/L

2. SUMMARY OF METHOD

2.1. A 1 mL aliquot of sample is diluted to a 40:60 methanol:water extract and analyzed by LC/MS/MS. PFAS are separated from other components on a C18 column with a solvent gradient program using 20mM ammonium acetate/water and methanol.

3. DEFINITIONS

Refer to Section 3 of the main body of this SOP for a summary of definitions.

4. INTERFERENCES

Refer to Section 4 of the main body of this SOP for interferences.

5. SAFETY

Refer to Section 5 of the main body of this SOP for safety information.

6. EQUIPMENT AND SUPPLIES

Refer to Section 6 of the main body of this SOP for supplies, other than those listed below specific to the in line SPE analysis.

6.1. 2 mL auto sampler vials, clear glass, Thermo Scientific Nation surestop vial, part no. C5000-1, or equivalent.

6.2. Vial caps, Thermo Scientific National AVCS blue cap, pre slit TEF/STL septa, part no. C5000-55B or equivalent.

6.3. Eppendorf 1000 uL epTIPS, part no. 022491954 or equivalent.

6.4. Eppendorf 200 uL epTIPS, part no. 022491938 or equivalent.

6.5. 50 mL graduated plastic centrifuge tubes, SCP Science DigiTUBES part no. 010-500-263 or equivalent.

6.6. 1000 uL Pipette: Eppendorf Research Plus.

6.7. 100 uL Pipette: Rainin EDP3-Plus.

6.8. 250 mL HDPE bottles with PPE screw caps, ESS part no. 0250-1902-QC or equivalent.

6.9. Analytical columns

6.9.1. Phenomenex Gemini C18 3 um, 3.0 mm x 100 mm, Part No. 00D-4439-Y0, or equivalent.

6.9.2. PFAS Isolator column, Phenomenex Luna C18 5 um, 50 mm x 4.6 mm, part no. 00B-4252-E 0 or equivalent.

6.10. SCIEX 5500 Triple Quad MS. The system utilizes Chrom Peak Review, version 2.1 or equivalent.

6.11. Shimadzu CTO-20AC HPLC equipped with 3 LC-20AD pumps and one DGU-20 degassing unit or equivalent.

7. REAGENTS AND STANDARDS

Refer to Section 7 of the main body of this SOP for reagents and standards, other than those listed below specific to the in line SPE analysis.

7.1. Reagent grade chemicals shall be used in all tests whenever available. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on the Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.1.1. Ammonium acetate, Fisher Optima LCMS grade (20 mM in water), part no. A114-50, or equivalent.

7.1.2. Methanol, Baker HPLC grade, part no. 9093-03.

7.1.3. Water, Nanopure or Millipore or Fisher Optima LCMS grade, part no. W6-4, must be free of interference and target analytes.

7.2. Calibration Standards

The calibration stock solution is prepared by diluting the appropriate amounts of the stock solutions (Section 7.2 of the main body of this SOP) in 40:60 methanol:water. The calibration stock solution is diluted with methanol to produce initial calibration standards. These are the normal calibration levels used. A different range can be used if needed to achieve lower reporting limits or a higher linear range.

7.3. Initial Calibration (ICAL) Levels (ng/L)

Compound	**CS-1**	**CS-2**	**CS-3**	**CS-4**	**CS-5**	**CS-6**	**CS-7**	**CS-8**
Perfluoroalkylcarboxylic acids (PFCAs)								
PFHpA	1.0	2.0	5.0	10	20	50	100	200
PFOA	1.0	2.0	5.0	10	20	50	100	200
PFNA	1.0	2.0	5.0	10	20	50	100	200
Perfluorinated sulfonic acids (PFSAs)								
PFBS	1.0	2.0	5.0	10	20	50	100	200
PFHxS	1.0	2.0	5.0	10	20	50	100	200
PFOS	1.0	2.0	5.0	10	20	50	100	200
Labeled Isotope Dilution Analytes (IDA)								
13C4-PFHpA	50	50	50	50	50	50	50	50
13C4-PFOA	50	50	50	50	50	50	50	50
13C5-PFNA	50	50	50	50	50	50	50	50
18O2-PFHxS	50	50	50	50	50	50	50	50
13C4-PFOS	50	50	50	50	50	50	50	50
13C3-PFBS	50	50	50	50	50	50	50	50

Note: *The above calibration levels are provided only as an example. The actual ICAL level used for each analytical batch will depend upon the LOQ requirements of the program.*

7.4. LCS/Matrix PFC Spike Solution, 100 ng/mL.

The PFC spike solution is prepared by diluting all PFAS to produce a solution containing each PFAS at 100 ng/mL in methanol.

7.5. PFC Isotope Dilution Analyte (IDA) Spike Solution, 1 ng/mL.

The PFC-IDA solution is prepared by diluting all labeled PFAS to produce a solution containing each at 1 ng/mL in methanol.

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1. Water samples are collected in pre-cleaned 250 mL HDPE containers. Other containers may also be suitable. Samples are chilled to 0 - 6 °C for shipment to the laboratory.

8.2. Samples are logged in following normal laboratory procedures and are stored under refrigeration at 0 - 6 °C. Water samples must be analyzed within 28 days of collection.

9. QUALITY CONTROL

Refer to Section 9 of the main body of this SOP for Quality Control information.

9.1. If potable water samples from the state of New York (NY) are analyzed via this method the control limits for LCS and IDA for PFOS and PFOA recoveries are 70-130%. If these limits are not met, refer to Section 9 of the main body of this SOP for corrective action.

9.2. If POST (treatment) samples have positive detections, review the associated PRE and MID (treatment) samples for similar detections. Re-preparation and re-analysis may be needed.

9.3. If PFBS is detected in the method blank greater than the RL, evaluate data for impact. PFBS is a known laboratory artifact. Re-preparation and re-analysis may be needed.

10. CALIBRATION

Refer to Section 10 of the main body of the SOP for calibration information.

11. PROCEDURE

Refer to Section 11 of the main body of this SOP for procedures, other than those listed below specific to the in line SPE analysis.

11.1. Water Sample Preparation

11.1.1. Visually inspect samples for the presence of settled and or suspended sediment/particulate. Evaluate if the sample can be decanted or centrifuged; if not, contact the client for guidance. Filtering the sample can lead to a low bias.

If authorized by the client to filter the sample, filter the water sample through a glass fiber filter (Whatman GF/F Cat No 1825 090 or equivalent).

Gravity of vacuum can be used to pass the sample through the filter. Prepare a filtration blank with any samples requiring filtration. File an NCM noting the need for filtration.

Warning: The use of a vacuum system creates the risk of glassware implosion. Inspect all glassware prior to use. Glassware with chips, scratches, rub marks or cracks must not be used.

11.1.2. Prepare an LCS and method blank by adding 250 mL of HPLC grade water into a 250 mL HDPE bottle.

11.1.3. If requested, find the client assigned sample for MS/MSD.

11.1.4. Spike directly into the sample bottles for the LCS and MS/MSD (if requested) with 0.050 mL (50 uL) of the LCS/Matrix PFC Spike solution (Section 7.4). This will result in a sample concentration of 20 ng/L. Shake well to disperse spike.

11.1.5. Measure 1 mL of each sample using an Eppendorf pipette and pour into a labeled 2.0 mL injection vial. This includes the LCS and method blank samples as well.

11.1.6. Be sure to "prepare" the pipette by collecting two 1 mL aliquots and disposing of them, and then collect the aliquot for testing.

11.1.7. Add 83 uL of surrogate solution (PFC IDA Spike Solution, Section 7.5) into each vial for each sample and QC sample. This will result in an extract concentration of 50 ng/L for the surrogate.

11.1.8. Add 577 uL of methanol to each sample for a final solvent composition of 40:60 methanol:water.

11.1.9. Seal the vial with a polypropylene screw cap. Note: Teflon lined caps can not be used due to detection of low level concentration of PFAS.

11.1.10. Vortex to mix the mixture well.

11.2. Instrument Analysis

11.2.1. Suggested operation conditions are listed in Tables 1A-1C below:

Table 1A - Routine Instrument Operating Conditions	
HPLC Conditions (Shimadzu HPLC)	
Column (Column temp = 35°C)	Phenomenex Gemini C18 3 um, 3.0 mm x 100 mm
Mobile Phase Composition	A = 20 mM Ammonium Acetate in Water B = Methanol

Table 1A - Routine Instrument Operating Conditions					
HPLC Conditions (Shimadzu HPLC)					
Gradient Program	**Time (min)**	**%A**	**%B**	**Curve**	**Flow Rate (mL/min)**
	0	90	10	6	0.60
	1	90	10	6	0.60
	1.5	35	65	6	0.60
	8	5	95	6	0.60
	8.1	1	99	6	0.60
	12	1	99	6	0.60
	12.5	90	10	6	0.60
	Maximum Pressure limit = 5,000 psi				
Injection Size	950 uL (fixed amount throughout the sequence)				
Run Time	17.1 minutes				
MS Interface Mode	ESI Negative Ion. Minimum of 10 scans/peak.				
Ion Spray Voltage (kV)	4.5				
Entrance Potential (V)	5				
Declustering Potential (V)	25				
Desolvation Temp	550 ºC				
Curtain Gas (nitrogen) Flow	35 psi				
Collision Gas (nitrogen) Flow	8 psi				

Table 1B - Routine Instrument Operating Conditions						
Mass Spectrometer Scan Settings (SCIEX 5500)						
Compound	**Comments**	**Reaction (MRM)**	**Dwell (sec)**	**Ent. Pot. (V)**	**Col. Energy (V)**	**Declu. Pot. (V)**
PFBS	Perfluorobutanesulfonate	299 > 80	0.02	6	58	55
13C3-PFBS	IDA	302 > 83	0.02	12	74	60
PFHpA	Perfluoroheptanoic acid	363 > 319	0.02	6	12	25
13C4-PFHpA	IDA	367 > 322	0.02	6	12	25
PFHxS	Perfluorohexanesulfonate	399 > 80	0.02	12	74	60
18O2-PFHxS	IDA	403 > 84	0.02	12	74	60
PFOA	Perfluorooctanoic acid	413 > 369	0.02	6	14	25
13C4PFOA	IDA	417 > 372	0.02	6	14	25
PFNA	Perfluorononanoic acid	463 > 419	0.02	6	14	25
13C5-PFNA	IDA	468 > 423	0.02	6	14	25
PFOS	Perfluorooctanesulfonate	499 > 80	0.02	9	108	65
13C4-PFOS	IDA	503 > 80	0.02	9	108	65

Table 1C				
Native Compounds	**Typical Native RT (minutes)**	**IDA analog**	**Typical IDA RT (minutes)**	**Quantitation Method**
PFBS	6.68	13C3-PFBS	6.68	Isotope Dilution
PFHpA	7.77	13C4-PFHpA	7.77	Isotope Dilution
PFHxS	7.76	18O2-PFHxS	7.76	Isotope Dilution
PFOA	8.44	13C4-PFOA	8.44	Isotope Dilution
PFNA	9.10	13C5-PFNA	9.10	Isotope Dilution
PFOS	9.06	13C4-PFOS	9.06	Isotope Dilution

11.2.2. Tune and calibrate the instrument as described in Section 10.

11.2.3. A typical run sequence is as follows:

- Primer (A number of primers are injected for conditioning of the instrument before analysis, especially when the instrument was idled or changed from a different analysis).
- Blank
- Calibration Curve
- ICB
- ICV
- PFOA RT marker (as needed)
- Rinse Blank (RB, not linked to anything)
- MB
- LCS
- LCSD (if applicable)
- Sample 1
- Sample 1 MS (if applicable)
- Sample 1 MSD (if applicable)
- Sample 2 (up to sample 10 before next CCV)
- CCV
- Up to 10 samples.
- End sequence with CCV

12. CALCULATIONS

Refer to Section 12 of the main body of this SOP for calculation information.

13. METHOD PERFORMANCE

Refer to Section 13 of the main body of this SOP for method performance information.

14. POLLUTION PREVENTION

Refer to Section 14 of the main body of this SOP for pollution prevention information.

15. WASTE MANAGEMENT

Refer to Section 15 of the main body of this SOP for waste management information.

16. REFERENCES

Refer to Section 16 of the main body of this SOP for reference information.

17. METHOD MODIFICATIONS

17.1. Refer to Section 17 of the main body of this SOP for modifications from Method 537, except as detailed below:

17.1.1. Water samples are prepared at 1.0 mL, not 250 mL.

17.1.2. Water sample containers are not preserved with Trizma. Holding time has been changed to 28 days for analysis.

17.1.3. The eluents and HPLC configuration differs. As a result the final extract is in 40:60 methanol:water.

18. ATTACHMENTS

There are no attachments to this Appendix.

19. REVISION HISTORY

Revisions prior to 04/10/2017 have been removed and are available in previous versions of this SOP.

19.1. WS-LC-0025, Attachment 1, Revision 3.5, Effective 02/27/2019

19.1.1. No changes to the attachment with this revision.

19.2. WS-LC-0025, Attachment 1, Revision 3.4, Effective 02/13/2019

19.2.1. Removed Section 3.6, “MPFOA: Perfluoro-n-[1,2,3,4-13C4]octanoic acid. Carbon-13 labeled PFOA”.

19.2.2. Removed Section 3.7, “MPFOS: Perfluoro-1-[1,2,3,4-13C4]octanesulfonic acid. Carbon-13 labeled PFOS”.

19.2.3. Section 7.2.3 removed, “MPFOS”.

19.2.4. Section 7.3 removed, “PFCA and PFSA”.

19.2.5. Section 7.3 added “13C3-PFBS” entry to table.

19.2.6. Section 10.11.3 revised to, “Projects performed under the auspices of the DoD/DOE QSM (Version 5.1) and the state of New Jersey must meet these criteria for the ICV: Analyte concentrations must be within ±30% of their true values for all analytes, IDA and target.”

19.2.7. Table 1B, revised PFBS IDA from “18O2-PFHxS” to “13C3-PFBS” and updated entry values.

19.2.8. Table 1C, revised “IS Analog” to “IDA Analog”, revised the PFBS IDA from “18O2-PFHxS” to “13C3-PFBS”, and updated entry values.

19.2.9. Editorial changes.

19.3. WS-LC-0025, Attachment 1, Revision 3.3, Effective 12/03/2018

19.3.1. No changes to the attachment with this revision.

19.4. WS-LC-0025, Attachment 1, Revision 3.2, Effective 08/20/2018

19.4.1. No changes to the attachment with this revision.

19.5. WS-LC-0025, Attachment 1, Revision 3.1, Effective 06/21/2018

19.5.1. No changes to the attachment with this revision.

19.6. WS-LC-0025, Attachment 1, Revision 3.0, Effective 04/13/2018

19.6.1. Updated labeling and formatting of Tables 1A-1C.

19.6.2. Added section 11.2.3, detailing a typical run sequence.

19.7. WS-LC-0025, Attachment 1, Revision 2.9, Effective 11/27/2017

19.7.1. No changes to the attachment with this revision.

19.8. WS-LC-0025, Attachment 1, Revision 2.8, Effective 11/06/2017

19.8.1. Section 11.2.1, Routine Instrument Operating Conditions table (SCIEX 5500), added “Minimum of 10 scans/peak”.

19.9. WS-LC-0025, Attachment 1, Revision 2.7, Effective 09/22/2017

19.9.1. Section 6.5, removed "The 5 items above are to be maintained in the drawer labeled "Segregated Supplies for in line SPE Analysis" in the LC/MS instrument room."

19.9.2. Added Sections 9.1 – 9.3.

19.9.3. Updated Section 11.1.

19.9.4. Editorial changes.

19.10. WS-LC-0025 Attachment 1, Revision 2.6, Effective 08/11/2017

19.10.1. No revisions to this attachment.

19.11. WS-LC-0025 Attachment 1, Revision 2.5, Effective 07/10/2017

19.11.1. No revisions to this attachment.

19.12. WS-LC-0025 Attachment 1, Revision 2.4, Effective 04/25/2017

19.12.1. No revisions to this attachment.

19.13. WS-LC-0025 Attachment 1, Revision 2.3, Effective 04/10/2017

19.13.1. Changed all mentions of "direct aqueous injection (DAI)" to "in line solid phase extraction (SPE)."

19.13.2. Inserted Section 17.1, and changed formatting of the modifications to Method 537 to Section 17.2 and subheadings.